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Effect of transportation and freeze-thaw procedure on hemostatic tests
Donma-çözülme ve taşıma prosedürünün hemostatik testlere etkisi

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Abstract

Objective: Coagulation tests are sensitive to pre-analytical variables. The aim of our study is to identify the effect of transportation and freeze-thaw status on for Factor VIII, Factor IX, Anti-thrombin III, Protein S, Protein C, Pro-thrombin time (PT) and Activated partial thromboplastin time (aPTT).

Materials and methods: The study was performed on 102 plasma samples obtained from 34 healthy volunteers. The samples were divided into three groups. Group A was analyzed whereas group B, C were frozen at −20°C. After 24 h, group B and C were transported for 2 h. Following the transfer, group B was analyzed and C was frozen at −20°C. After 24 h, group C was analyzed. Analyses of samples were performed in Thrombolyzer-XRM for PT, aPTT, Factor VIII, Factor IX, Anti-thrombin III, Protein C and Protein S.

Results: There were significant variations for PT, aPTT, Protein S, Factor VIII and Factor IX for group A&B and A&C comparisons in different stability criteria approaches. In significant change limit and percentage change calculations Protein S, Factor VIII and IX showed significant differences. For acceptable change limit approach, aPTT and Factor IX showed significant changes.

Conclusion: Laboratories should take precautions for transportation and freeze-thaw cycles to prevent inaccu-rate results.

Keywords: Coagulation tests; Freeze-thaw; Frozen sample; Transportation; Pre-analytical variables.

Öz

Amaç: Koagulasyon testleri analiz öncesi değişkenlere karşı duyarlıdır. Çalışmamızın amacı, donma-çözünme ve taşıma etkisinin Faktör VIII, Faktör IX, Anti-trombin III, Protein S, Protein C, Protrombin zamanı (PT) ve Aktive parsiyel thromboplastin zamanı (aPTT) sonuçlarına etkisini araştırmaktır.

Gereç ve Yöntem: Çalışma 34 sağlıklı gönüllüden elde edilen 102 plazma örneğinde gerçekleştirilmiştir. Örnekler 3 gruba ayrıldı: Grup A örnekleri hemen analiz edildiğinde, Grup B ve Grup C örnekleri −20°C'de donduruldu. 24 saat sonra Grup B ve Grup C örnekleri 2 saat süre boyunca taşıtıldı. Taşımanın ardından, Grup B örnekleri analiz edildiğinde, Grup C örnekleri tekra −20°C'de donduruldu. 24 saat sonra Grup C örnekleri analiz edildi. Örneklerden Thrombolyzer-XRM cihazında, PT, aPTT, Faktör VIII, Faktör IX, Anti-trombin III, Protein C ve Protein S testleri gerçekleştirildi.

Sonuçlar: Farklı stabilite kriterleriyle analiz edilmiş gözlemlerden PT, aPTT, Protein S, Faktör VIII ve Faktör IX sonuçlarında Grup A&B ve A&C karşılaştırılardan önemli farklılıklar elde edildi. Anlamlı değişim sırrını ve yüzde değişim hesaplamalarında Protein S, Faktör VIII ve Faktör IX sonuçlarında, kabul edilebilir değişim sırrını yakalamak için ise aPTT ve Faktör IX sonuçları anlamalı farklılıklar bulundu.

Tartışma: Laboratuvarlar yanlış sonuçları önlemek için örneklerin taşıınma ve donma-çözünme döngüleri için önlemler almalıdır.
Anahtar kelimeler: Koagulasyon testleri; donma ve çözünme; donmuş örnek; preanalitik değişkenler; taşınma.

Introduction

Coagulation tests are sensitive to pre-analytical variables such as patient related factors, phlebotomy practices, sample collection, transportation and handling, coagulation tubes, centrifugation, storage time, temperature and assay method [1–4]. Pre-analytical variables have been defined as the main cause of all laboratory errors [5–7]. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests are usually performed in every clinical laboratory, whereas specific tests such as Factor VIII, Factor IX, Anti-thrombin III, Protein S or Protein C are usually sent to tertiary, core or central laboratories due to inefficiency of the tests, financial burden, technical disadvantages or insufficient test request. Once the tests are ordered, samples are centrifuged to obtain plasma after phlebotomy and then are frozen before transportation to stabilize specific coagulation test parameters.

Transport conditions are crucial for the quality of the coagulation test results [1]. During transportation, even limited alterations in temperature, humidity, physical force or sample position may alter coagulation test results [1, 8]. Safety transport containers are designed to preserve inner environment of the box against the changes the outside of the container [9]. These containers are used for storage and transportation of laboratory samples such as plasma, serum, urine or other biological materials.

It is important to evaluate the effect of transport on the samples, taking into account the common necessity of the shipping of the specific coagulation tests and the lack of standardization in the transportation practice such as temperature range of inside of the containers for frozen samples or transportation time. CLSI H21-A5 suggests that transport to the processing site should be completed within an hour [10]. Harrison et al. [11] and Mackie et al. [12] suggested that samples should be shipped non/refrigerated at 15–25°C in a short time as possible but in practice local laboratories often fail to complete the process on time. There are limited studies that evaluate the effect of transportation on coagulation test results [13] but there is no study which investigates the effect of transportation and freeze-thaw status on frozen samples for specific coagulation tests. The aim of our study is to identify the effect of transportation and freeze-thaw status on for Factor VIII, Factor IX, Anti-thrombin III, Protein S and Protein C, PT and aPTT.

Materials and methods

Blood donors and blood sampling

This study was approved by the ethics board of Maltepe University (issue number 2017/900/99) and blood sampling took place at Maltepe University Education and Research Hospital Central Laboratory. The study included 36 healthy volunteers (physicians, nurses, laboratory technicians and university students) without history of any serious medical conditions and who were not under any medication. Seventeen of the volunteers were male and 19 were female. Written informed consents were obtained from all individual participants.

Blood samples were obtained between 8 and 10 am after a 12-h fasting period using hospital’s standard venipuncture operating procedures. Three tubes of blood from each individual were taken into 1.8 mL evacuated tubes containing 3.2% buffered sodium citrate (BD Vacutainer, UK) at a blood-to-anticogulant ratio of 9:1. The results were valid for BD vacutainer tubes in our study. All samples from all individuals were centrifuged for 15 min at 1500 g at room temperature to obtain fresh plasma. Two samples were rejected due to hemolysis; therefore, the study was performed on 102 plasma samples obtained from 34 healthy volunteers. Fresh plasma samples from three tubes were mixed in a different empty tube. Three aliquots were allocated into different plastic tubes for three different time period. Samples were analyzed for the most requested coagulation test parameters which are PT, aPTT, Factor VIII, Factor IX, Anti-thrombin III, Protein C and Protein S for clinicians.

Study design

The samples obtained from the volunteers were divided into three groups. First group of samples (group A) were analyzed immediately to get basic results that were considered to be the most optimum/accurate. Fresh samples from 34 volunteers were immediately analyzed for PT, aPTT, Factor VIII, Factor IX, Anti-thrombin III, Protein C and Protein S. Second group samples (group B) were frozen immediately at −20°C. After 24 h, the samples were transported for 2 h. A double covered foam thermal box was used during the transport simulation. Following the transfer, the samples were immediately analyzed for the same homeostatic parameters analyzed in Group A. The third group of samples (group C) were immediately frozen.
at −20°C. On the next day, the samples were transported for 2 h (the same transport simulation). Following the transport, they were re-frozen at −20°C. After 24 h, the samples were analyzed for the same coagulation parameters (Figure 1).

A calibrated temperature recorder (Hygro Thermometer) was inserted into the transport box. The temperatures of the container, vehicle and outside the vehicle were all monitored every 10 min for 2 h. Samples were taken back to the same laboratory after transport simulation was completed (Figure 2).

**Figure 1:** Algorithm of blood samplings for three different groups. Group A were analyzed immediately. Group B were frozen immediately at −20°C. After 24 h, the samples were transported for 2 h. Following the transfer, the samples were immediately analyzed. Group C were immediately frozen at −20°C. On the next day, the samples were transported for 2 h. Following the transport, they were re-frozen at −20°C. After 24 h, the samples were analyzed for the same coagulation parameters.

**Figure 2:** Temperature recordings during transportation simulation. The temperatures of the container, vehicle and outside the vehicle were all monitored every 10 min for 2 h.

**Analysis procedure**

Analyses of all samples for three groups were performed in Thrombolyzer XRM (Norderstedt Germany). Plasma Factor VIII, Factor IX, Protein S and aPTT levels were measured by a Hyphen Biomed kit (Neuville sur Oise France) using a clotting method, Protein C and Anti-trombin III levels were determined by Hyphen Biomed kit (Neuville sur Oise France) using a chromogenic method and PT levels were measured by a BIO-TP LI kit (Maizy France) using a clotting method. Within run precision values were calculated as coefficient of variation (CV%) 1.0, 1.4, 2.2, 7.1, 8.0, 8.9 and 1.51 for PT, aPTT, Protein C, Protein S, Factor VIII, Factor IX and Anti-thrombin III, respectively. Between day CV values of low control plasma were measured as 3.8, 2.4, 5.2, 8.9, 5.1, 6.8, 5.7 and between day CV values of high control plasma were measured as 1.8, 2.5, 4.7, 8.8, 8.4, 8.1, 5.8 for PT, aPTT, Protein C, Protein S, Factor VIII, Factor IX and Anti-thrombin III respectively (Table 1).

**Statistical analysis**

Statistical analyses were performed using SPSS version 21 (SPSS, Chicago, IL, USA) and p value <0.05 was considered statistically significant. There are different stability criteria on the aspect of researcher’s approach for advanced or routine coagulation tests. We considered five of these approaches to evaluate sample stability:

1. **Percentage CV change:** Imeri et al. [14] defined that biological sample stability can be evaluated as average change smaller than one percentage CV variation of the method.

2. **Mean percentage change:** In some coagulation studies, mean >10% percentage change for comparisons was considered significant as the stability criteria [15–17]. In addition, ±5% and ±10% variations were used as stability criteria of coagulation tests in CLSI H21-A5 document [10]. Percentage change was calculated by [result of group B or C-result of group A]/result of group A ×100.

3. **Acceptable Change Limit (ACL)** [18]: ACL was calculated by 2.77*CV of the analytic method. A mean percentage change greater than ACL indicated a probable difference in analyte concentration. The CV was achieved from the data of QC values.

4. **Paired samples t-test** [19, 20]: Group A&B and A&C were compared and p value <0.05 was considered statistically significant.

5. **Appointment of clinically significant changes by Significant Change Limit (SCL) approach** [21, 22]: SCL was
calculated by “Initial value ± 2.8*standard deviation”
In our study, mean values of Group A was defined as initial value.

The groups were also re-analyzed for correlation point of view with Pearson correlation of coefficient for the ones that had different results according to aforementioned five approaches. The strength of correlation for the absolute r values were considered as 0–0.19 “very weak”, 0.20–0.39 “weak”, 0.40–0.59 “moderate”, 0.60–0.79 “strong” and finally 0.80–1.0 “very strong” [23].

**Results**

CV values of quality controls have been demonstrated in Table 1. All parameters, except Anti-thrombin III, showed significant changes according to percentage CV change approach. This approach was excluded because the CV values of quality controls were very low for PT, aPTT and Protein C. In group A and group B comparisons, there were 2.7%, 7%, 9.5%, 12.4%, 27.5% and 4.5% variations for PT, aPTT, Protein C, Protein S, Factor VIII, Factor IX and Anti-thrombin III tests, respectively. In group A and C comparisons there were 3.8%, 7.1%, 7.8%, 12.7%, 13.2%, 26.4% and 4.3% variations for PT, aPTT, Protein C, Protein S, Factor VIII, Factor IX and Anti-thrombin III tests, respectively (Table 1). There were significant differences for aPTT in group A&B and group A&C and for Factor IX in group A&B and A&C according to ACL approach (Table 1). There were statistically significant changes for PT, aPTT, Protein S, Factor VIII and Factor IX in both group A&B and group A&C comparisons when paired samples t-test were applied (Table 1). There were significant differences for Protein S in group B, for Factor VIII in group B and C, for Factor IX in group B and C according to SCL approach (Table 2). According to these aforementioned approaches to evaluate sample stability, significant changes were determined for PT, aPTT, Protein S, Factor VIII and Factor IX tests in both group A&B and A&C comparisons.

Those aforementioned groups were evaluated for correlation for PT, aPTT, Factor VIII, Factor IX and Protein S. For PT measurements there were very strong correlations between groups A&B (r = 0.95), strong correlations between groups A&C (r = 0.77) and B&C (r = 0.69) (Figure 3 A1, A2 and A3). For aPTT measurements there were very

| Test          | % Change between group A and B | % Change between group A and C | CV of quality controls | ACL = CV Value × 2.77 | Paired samples t-test Between group A and B | Paired samples t-test Between group A and C |
|---------------|--------------------------------|--------------------------------|------------------------|------------------------|---------------------------------------------|---------------------------------------------|
| PT            | 2.7                            | 3.8                            | 1.8                    | 4.9                    | p < 0.05                                    | p < 0.05                                   |
| aPTT          | 7.0<sup>a</sup>                 | 7.1<sup>a</sup>                | 2.5                    | 6.9                    | p < 0.05                                    | p < 0.05                                   |
| Protein C     | 9.5                            | 7.8                            | 4.7                    | 13.1                   | Not significant                             | Not significant                             |
| Protein S     | 17.2<sup>a,b</sup>             | 12.7<sup>b</sup>               | 8.8                    | 24.2                   | p < 0.05                                    | p < 0.05                                   |
| Factor VIII   | 12.4<sup>a,b</sup>             | 13.2<sup>b</sup>               | 8.4                    | 23.4                   | p < 0.05                                    | p < 0.05                                   |
| Factor IX     | 27.5<sup>a</sup><sup>,b</sup>  | 26.4<sup>a</sup><sup>,b</sup>   | 8.1                    | 22.5                   | p < 0.05                                    | p < 0.05                                   |
| Anti-thrombin III | 4.5                  | 4.3                            | 5.8                    | 16.1                   | Not significant                             | Not significant                             |

<sup>a</sup>Shows significant differences according to ACL. <sup>b</sup>Shows higher than 10% change.

**Table 2:** Significant Change Limit (SCL)’s of the whole parameters and their mean values.

| Test          | Mean values of group A | Mean values of group B | Mean values of group C | SCL range | SCL (SD × 2.8) |
|---------------|------------------------|------------------------|------------------------|-----------|----------------|
| PT            | 12.8                   | 13.0                   | 13.0                   | 11.4–14.2 | 0.5 × 2.8 = 1.4 |
| aPTT          | 26.5                   | 28.0                   | 28.2                   | 24.5–28.5 | 0.7 × 2.8 = 2.0 |
| Protein C     | 109.6                  | 112.7                  | 112.5                  | 104.6–114.6| 1.8 × 2.8 = 5.0 |
| Protein S     | 80.9                   | 71.1<sup>a</sup>       | 73.3                   | 71.9–89.9 | 3.2 × 2.8 = 9.0 |
| Factor VIII   | 118.3                  | 107.1<sup>a</sup>      | 106.5<sup>a</sup>      | 112.4–124.2| 2.1 × 2.8 = 5.9 |
| Factor IX     | 137.4                  | 97.6<sup>a</sup>       | 102.1<sup>a</sup>      | 123.7–151.1| 4.9 × 2.8 = 13.7 |
| Anti-thrombin III | 93.2                  | 93.0                   | 92.2                   | 87.3–99.1 | 2.1 × 2.8 = 5.9 |

<sup>a</sup>Shows significant differences according to SCL.
strong correlations between groups A&B ($r = 0.81$), A&C ($r = 0.88$) and B&C ($r = 0.91$) (Figure 3 B1, B2 and B3). For Protein S measurements there were moderate correlation between groups A&B ($r = 0.55$), strong correlation between groups A&C ($r = 0.72$) and strong correlation between groups B&C ($r = 0.64$) (Figure 3 C1, C2 and C3). For Factor

Figure 3: Pearson correlations of affected groups for PT, aPTT, Factor VIII, Factor IX and Protein S. For PT measurements there were very strong correlations between groups A&B, strong correlations between groups A&C and B&C. For aPTT measurements there were very strong correlations between groups A&B, A&C and B&C. For Protein S measurements there were moderate correlation between groups A&B, strong correlation between groups A&C and groups B&C. For Factor VIII measurements, there were very strong correlations between groups A&B, A&C and B&C. For Factor IX measurements there were moderate correlations between groups A&B, A&C and B&C. For Protein S measurements there were moderate correlation between groups A&B ($r = 0.55$), strong correlation between groups A&C ($r = 0.72$) and strong correlation between groups B&C ($r = 0.64$) (Figure 3 C1, C2 and C3). For Factor
VIII measurements, there were very strong correlations between groups A&B ($r=0.83$), A&C ($r=0.88$) and B&C ($r=0.90$) (Figure 3 D1, D2 and D3). For Factor IX measurements there were moderate correlations between groups A&B ($r=0.44$), A&C ($r=0.42$) and B&C ($r=0.59$) (Figure 3 E1, E2 and E3).

**Discussion**

Hospitals and/or local laboratories may not perform some specific laboratory tests due to financial, time related or technical problems. Tertiary reference or central laboratories usually measure specified advanced coagulation test parameters once or twice a week depending on their work and patient load. In order to accomplish this duty, samples are generally transferred at the same day of phlebotomy or frozen in deep freeze until analyzed another day. According to traditional procedures, samples are dispatched at room temperature, or at 2–8°C or frozen during the transport to the core laboratories depending on the type of specimen. However, unfortunately there is lack of evidence-based reference at the current medical literature explaining temperature range, humidity, thermal protection or transport duration for the transportation procedure. It has been known that transport containers are critical for thermal insulation during shipment. Lippi et al. investigated compatibility of a transport container for blood sample shipment at 2–8°C [9]. They demonstrated that temperature stability was sufficient during the shipment time before 120th min but insufficient from 120th to 450th min. Pottengieier et al. investigated pre-analytical stability of blood samples consisting of 21 test parameters including blood cells, coagulation tests (aPTT, INR, Anti-thrombin III and Fibrinogen), electrolytes and markers of cardiac ischemia [26]. They analyzed one sample set immediately whereas the other set was analyzed 60 min later. They showed that tested parameters were generally within clinically acceptable ranges. Zaninotto et al. evaluated effects of sample transportation on commonly requested laboratory tests such as alanine aminotransferase, aPTT, calcium, potassium, etc. [25]. They compared the sample test results obtained from peripheral centers in 2007 to samples collected in 2011 after introduction of an integrated transportation system including a container and a temperature recorder. They reported significant variations for aPTT test results between short and long term transportations in 2007. After introduction of their own and special transport system, variations were minimal. Although Zaninotto et al. emphasized the quality improvement of sample shipping with an integration of a transportation system, there is no study which evaluates the effect of shipment directly on frozen samples in the literature.

There are some studies evaluating the effects of storage time and temperature on coagulation tests. Bach et al. [26] found that freezing and thawing induces to a significantly decrease in F VIII activity. Zhao et al. [27] investigated the impact of pre-analytical frozen storage time and temperature on aPTT, fibrinogen, PT/INR, thrombin time, factor VIII and factor IX activity. 144 samples were divided into four groups; stored at −80°C or −20°C and analyzed by CS5100 or CA7000. They compared the baseline results with storage for 15 days, 1 month, 3 months, 6 months, 1 year. They found that PT/INR, fibrinogen, and thrombin time can be stored for 1 year, aPTT for 6 months, factor VIII and factor IX activity for 1 month at −80°C; fibrinogen and thrombin time can be stored for 1 year, PT/INR and factor IX activity for 1 month, aPTT and factor VIII activity for 15 days at −20°C. Feng et al. [16] examined the impacts of storage time and temperature on aPTT, PT, INR, fibrinogen, thrombin time, factor VIII and factor IX activity in 72 samples. They compared the baseline values with storage for 2, 4, 6, 8, 12 and 24 h at 25°C and 4°C in two centers. Their data revealed that PT, thrombin time and fibrinogen can be stored for ≤24 h; factor IX activity for ≤4 h; factor VIII activity for ≤2 h at 4°C and 25°C; aPTT for ≤12 h at 4°C and 8 h at 25°C.

Kim et al. [28] searched specimen stability on ice and room temperature for a broad panel of coagulation tests. One tube of whole blood was centrifuged immediately (time 0), one was stored for 4 h on ice, and one was stored for 4 h at room temperature before centrifugation. They found that there is no statistically significant differences for fibrinogen, activated protein C resistance, thrombin time, reptilase time, antithrombin activity, chromogenic protein C, factor XII, and antiplasmin activity among time 0, 4 h on ice, and 4 h at room temperature samples. But they found statistically, but not clinically, significant differences for PT, aPTT, factor IX, factor XI, protein S activity, and plasminogen activity.

There is only one study investigating the effect of transportation at room temperature for coagulation tests such as PT and aPTT. In this study, Ergin et al. [13] examined the impact of the transport of blood samples on coagulation tests such as PT and aPTT at room temperature. They compared the results from control group to that of centrifuged transported, noncentrifuged transported, centrifuged untransported and noncentrifuged untransported samples. Their data revealed that PT results from noncentrifuged transported samples were clinically
different compared to the control. In our study, no significant change was observed in the biostatistical analysis other than paired t-test for PT measurements. This finding can be considered consistent with the outcomes of Ergin et al.’s study. However, it is noteworthy that the percentage change, SCL and ACL values of current study were all within limits.

CLSI H21-A5 suggests that transport to the processing site should be completed within an hour [10]. PT assay transportation can be delayed with a centrifuged or uncentrifuged unopened tube up to 24 h from the time of specimen collection, for aPTT assay this time limit is only 4 h. If the test is not completed within 24 h for PT and within 4 h for aPTT and other assays, plasma should be stored −20°C or below up to 2 weeks or −70°C or below for long term storage [10]. But there is no transportation data for Protein C, Anti-thrombin III, Protein S, Factor VIII and Factor IX at current literature. Our data showed that the 2-h transport did not have any significant effect on the results of Protein C and Anti-thrombin III assays in all groups tested.

Protein S, Factor VIII and Factor IX are known as labile coagulation proteins. Plasma is more stable for labile proteins at room temperature instead of 4°C or 37°C. Delays between sample collection, transportation or analysis can cause activation or in vitro degradation of this parameters [29, 30]. Based on these information, percentage change and SCL approaches showed quite similar results supporting pathophysiological roles of previous parameters (Tables 1 and 2). In addition to this, aPTT results showed significant changes in paired samples t-test comparison and ACL approach (Table 1). This finding is relevant to Factor VIII and Factor IX results of SCL, paired samples t-test together with percentage change. Because aPTT is generally used for the detection of intrinsic pathway and also these factors contributed formation of intrinsic tenase complex [31].

In our study, transportation and temperature change significantly affected the results for PT, aPTT, Protein S, Factor VIII and Factor IX assays. The overall transport effect was similar between groups B and C. Consistently, we found correlation between these groups, which showed high analytical accuracy and reproducibility of the analytical kits and the tool indirectly. Between day CV’s and within run precision of the kits were quite good (less than 10%), which supports our finding that transportation duration and/or temperature variance can affect the results (Table 1). Even all test results were in reference limits, the transport effect should still be minimized for all in house or reference validation procedures.

Type of the tube, container of the anticoagulant, general features of thermal bag, defrosting procedure of deep freezers, vibration of the samples, time limits of shipping, number of thaw-freeze cycles are all substantial candidates that can interfere the results. It is obvious that these properties vary from one laboratory to another one, which makes it difficult to establish a proper guideline for transportation.

Conclusion and future directions

This research concludes that Protein C and Anti-thrombin III parameters can be transported into another reference laboratory according to in-house validation regulations. PT, aPTT, Protein S, Factor VIII and Factor IX transportation issue (preventing temperature change and freeze-thaw cycle) requires further evaluations, transportation method validation and individual laboratory based in house rules. Sample size, absence of patients who are at edges of life (newborns and geriatrics), absence of individuals who have established hematological disorders, lack of an extra different measurement method, lack of extra other highly qualified comparative tools (especially transportation bag and transportation vehicle), addition of and extra medical laboratory and recruitment of patients who have different pharmacological status were limitations for this current study. These issues should be overcome with new and different studies which will recruit many peripheral and central laboratories in future. Studies that include high number of patients, different time periods for different transportation scenarios with different pathologies can enlighten the horizons for hematological parameters and add new regulations to shipment guidelines.

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References

1. Funk DM, Lippi G, Favaloro EJ, editors. Quality standards for sample processing, transportation, and storage in hemostasis testing. Seminars in thrombosis and hemostasis. New York: Thieme Medical Publishers, 2012.
2. Lippi G, Salvagno GL, Montagnana M, Lima-Oliveira G, Guidi GC, Favalaro EJ, editors. Quality standards for sample collection in coagulation testing. Seminars in thrombosis and hemostasis. New York: Thieme Medical Publishers, 2012.

3. Lawrence JB. Preanalytical variables in the coagulation laboratory. Lab Med 2003;34:49–57.

4. Adcock DM, Favalaro EJ, Lippi G. Critical pre-examination variables in the hemostasis laboratory and their quality indicators. Clin Biochem 2016;49:1315–20.

5. Aakre KM, Langlois MR, Watine J, Barth JH, Baum H, Collinson P, et al. Critical review of laboratory investigations in clinical practice guidelines: proposals for the description of investigation. Clin Chem Lab Med 2013;51:1217–26.

6. Abdollahi A, Saffar H, Saffar H. Types and frequency of errors during different phases of testing at a clinical medical laboratory of a teaching hospital in Tehran, Iran. N Am J Med Sci 2014;6:224.

7. Romero A, Cobos A, Gómez J, Muñoz M. Role of training activities for the reduction of pre-analytical errors in laboratory samples from primary care. Clin Chim Acta 2012;413:166–9.

8. Plebani M, Lippi G. Is laboratory medicine a dying profession? Blessed are those who have not seen and yet have believed. Clin Biochem 2010;43:939–41.

9. Lippi G, Lima-Oliveira G, Nazer SC, Moreira ML, Souza RF, Salvagno GL, et al. Suitability of a transport box for blood sample shipment over a long period. Clin Biochem 2011;44:1028–9.

10. Edition AG-F. CLSI document H21-A5. Wayne, PA. 2008. Link https://clsi.org/media/1399/h21a5_sample.pdf.

11. Harrison P, Mackie I, Mumford A, Briggs R, Lister R, Winter M, et al. Guidelines for the laboratory investigation of heritable disorders of platelet function. Br J Haematol 2011;155:30–44.

12. Mackie I, Cooper P, Lawrie E, Kitchen S, Laffan M, et al. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Int J Lab Hematol 2013;35:1–13.

13. Ergan M, Erdogan S, Akturk O, Erol E. The effects of transport by car on coagulation tests. Clin Chem Lab Med 2017;55:1943–7.

14. Imeri F, Herklotz R, Risch L, Arbetsleitner C, Zerlauth M, Risch A, et al. Stability of hematological analytes depends on the hematology analyser used: a stability study with Bayer Advia 120, Beckman Coulter LH 750 and Sysmex XE 2100. Clin Chim Acta 2008;397:68–71.

15. Zürcher M, Sulzer I, Barizzi G, Lämmlle B, Alberio L. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. Thromb Haemost 2008;99:416–26.

16. Feng L, Zhao Y, Zhao H, Shao Z. Effects of storage time and temperature on coagulation tests and factors in fresh plasma. Sci Rep 2014;4:3868.

17. van Geest-Daalderop JH, Mulder AB, Boonman-de Winter LJ, Hoekstra MM, van den Besselaar AM. Preanalytical variables and off-site blood collection: influences on the results of the prothrombin time/international normalized ratio test and implications for monitoring of oral anticoagulant therapy. Clin Chem 2005;51:561–8.

18. Oddo C, Lombard E, Portugal H. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clin Biochem 2012;45:464–9.

19. Kul S. Uygun istatistiksel test seçim kilavuzu/guideline for suitable statistical test selection. Plevra Bülteni 2014;8:26.

20. Berg B, Estborn B, Ttridge N. Stability of serum and blood constituents during mail transport. Scand J Clin Lab Invest 1981;41:425–30.

21. Passey R. Quality control for the clinical chemistry laboratory. Clinical chemistry: theory, analysis, correlation, 3rd ed. St Louis: CV Mosby Company, 1996:385–91.

22. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7.

23. Evans JD. Straightforward statistics for the behavioral sciences. Belmont, CA, USA: Thomson Brooks/Cole Publishing Co., 1996.

24. Pottenger J, Jess N, Harig F, Gall C, Schmidt J, Birkoß T. Can we rely on out-of-hospital blood samples? A prospective interventional study on the pre-analytical stability of blood samples under prehospital emergency medicine conditions. Scand J Trauma Resusc Emerg Med 2017;25:24.

25. Zaninotto M, Tassinato A, Padoan A, Vecchiato G, Pinato A, Sciacovelli L, et al. Effects of sample transportation on commonly requested laboratory tests. Clin Chem Lab Med 2012;50:1755–60.

26. Bach J, Haubelt H, Hellstern P. Sources of variation in factor VIII, von Willebrand factor and fibrinogen measurements: implications for detecting deficiencies and increased plasma levels. Thromb Res 2010;126:188–95.

27. Zhao Y, Feng G, Zhang J, Gong R, Cai C, Feng L. Effects of preanalytical frozen storage time and temperature on screening coagulation tests and factors VIII and IX activity. Sci Rep 2017;7:12179.

28. Kim Y, Lewandrowski KB, Lucien FA, Van Cott EM. The effects of transport temperature and time on routine and specialized coagulation assays. Blood Coagul Fibrin 2018;29:184–8.

29. Loeffen R, Kleinegris MC, Loubele ST, Pluijmen PH, Fens D, Van Oerle R, et al. Preanalytic variables of thrombin generation: towards a standard procedure and validation of the method. J Thromb Haemost 2012;10:2544–54.

30. Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 2016;14:49.

31. Hall JE. Guyton and Hall textbook of medical physiology e-Book. Philadelphia, PA, USA: Elsevier Health Sciences, 2015.