



Influence of temperature and storage duration on measurement of activated partial thromboplastin time, D-dimers, fibrinogen, prothrombin time and thrombin time, in citrate-anticoagulated whole blood specimens

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Coagulation tests, temperature, storage duration

SUMMARY

Introduction: We report the effect of temperature and storage duration on a range of haematological analytes: activated partial thromboplastin time (APTT), D-dimers, fibrinogen (Fbg), prothrombin time (PT) and thrombin time (TT). All analytes were measured using a Sysmex CA7000 System with SIEMENS reagents.

Methods: One hundred and sixty patients were divided into two groups: samples from group A (80 patients) were used to assess the effect of storage at room temperature (RT) (0, 4, 8 and 24 h), and samples from group B (80 patients) were used to determine the effect of storage at 4 °C. Percentage changes compared with baseline results ($T = 0$ result) were calculated, and clinically relevant differences were defined as a percentage change of >10%.

Results: Changes in APTT, D-dimer, Fbg, PT and TT results following 4, 8 and 24 h storage were statistically significant at RT and 4 °C. For D-dimer, Fbg, PT and TT at both RT and 4 °C, the mean percentage changes after all storage periods were <10%, but for APTT, the mean percentage change following 24 h storage was >10% and exceeded the analytical within-batch imprecision.

Conclusion: We demonstrate that a storage time interval up to 24 h for D-dimers, Fbg, PT, and TT, and 8 h for APTT at either RT or 4 °C is acceptable.

INTRODUCTION

activated partial thromboplastin time (APTT), D-dimers, fibrinogen (Fbg), prothrombin time (PT) and thrombin time (TT) measurements are routine coagulation tests used to assess pathological alterations of the haemostatic and coagulation systems and guide

clinical therapy. Pre-analytical factors including specimen collection, transportation, centrifugation, storage and assay method can all affect coagulation testing results [1]. In 1982, it was demonstrated that PT reduction in both normal and patients receiving coumadin therapy was time and temperature dependent, when samples were collected into borosilicate or

siliconized borosilicate tubes, resulting in incorrect adjustment of warfarin dosage [2–4]. Consequently, the Clinical and Laboratory Standards Institute (CLSI) H21-A5 recommended that specimens should be analysed within 24-h for PT and 4-h for APTT if stored at RT but did not recommend a storage time for refrigerated storage (2–8 °C); furthermore, they recommended that PT samples should not be refrigerated [5].

Many studies have proposed acceptable storage durations for routine coagulation tests at RT and refrigerated temperatures [6–9]. A range of storage times (2, 4, 6, 8 h) and temperatures (4 °C, 25 °C, >30 °C) have been studied with various criteria for acceptability. In addition to the factors already discussed, the coagulation factors, II, VII, IX, X, XI, antithrombin (AT), have been shown to be stable for 24 h at RT [9]. Other pre-analytical factors have been investigated, including mechanical agitation, which was shown to have a large effect on PT/INR results and centrifugation conditions which resulted in no clinically or statistically relevant differences [10]. There is good agreement that short storage times are acceptable, but as storage duration is extended, the consensus becomes less clear. Consequently, it has been suggested that each laboratory should establish its own acceptable storage times.

Our hospital is part of a comprehensive hospital network with more than three thousand beds, with a large number of specimens received that can lead to delays in sample processing and difficulty in achieving the CLSI H21-A5 standards for APTT measurement. We therefore evaluated storage time for coagulation tests based on the CLSI H21-A5 guidelines and other study reports, to identify acceptable storage temperatures and duration for our laboratory. We assessed APTT, D-dimers, Fbg, PT and TT, after 0, 4, 8 and 24-h storage at RT and 4 °C, respectively.

MATERIALS AND METHODS

Ethical approval

This study was approved by the ethics committee of the First Affiliated Hospital of Medical College at Zhejiang University, China.

Patients

One hundred and sixty-eight patients were admitted to various clinical departments in the first affiliated

hospital of Zhejiang University between August 2012 and November 2012. Eight patients were excluded as they received unfractionated heparin, coumarin-based oral anticoagulant therapy, or suffered from severe liver cirrhosis, which meant that in our hospital, these samples are sent to a separate ‘urgent’ laboratory and would always be processed without delay. One hundred and sixty patients (84 men and 76 women; median age, 57 years; range, 18–91 years) were ultimately included. All patients gave informed consent to the use of their blood samples for the project.

Assays

All whole blood samples were collected using venepuncture into 5 mL vacutainers containing 0.5 mL 0.109 M sodium citrate (Becton Dickinson, Franklin Lakes, NJ, USA) in the morning following a 12-h fast. Samples were sent to the laboratory, centrifuged (10 min, 3000 *g*) and measured immediately using the primary sample tubes. Immediately after analysis, samples were recapped and stored at RT or 4 °C. These routine coagulation tests covered low, normal and high parts of the analytical range. Obviously jaundiced, lipaemic or hemolysed specimens were excluded. APTT, Fbg, PT and TT were measured by coagulation method using a Sysmex CA7000 System (Sysmex, Kobe, Japan) using SIEMENS reagents (SIEMENS, Marburg, Germany): Dade Actin activated cephaloplastin reagent (lot 547165), Dade Thrombin reagent (lot 538049A), Thromborel S (lot 545483) and Test Thrombin reagent (lot 504449), respectively. D-dimer was measured by immune turbidimetry method using a Sysmex CA7000 System using SIEMENS reagents: Innovance D-Dimer (lot 560152).

Two mixed pools having low and high target results were prepared from freshly collected plasma and were used to measure PT, APTT, Fbg, TT and D-dimer 20 times in 2 h to assess within-batch imprecision. Commercial quality control products (SIEMENS) with target results in normal/abnormal ranges were used to measure PT, APTT, Fbg, TT and D-dimer on 20 separate days to calculate between-batch imprecision.

The patient specimens were divided into two groups, group A (80 patients) to assess storage duration (0, 4, 8 and 24 h) at RT and group B (80 patients) to assess storage at 4 °C. The first (0 h) results were defined as baseline. Results were expressed in seconds (PT, APTT and

TT), g/L (Fbg) and µg/L fibrinogen equivalent units (FEU, D-dimer). Local laboratory-defined reference ranges based on CLSI C28-A2 guidelines [7] were as follows: 22.0–36.0 s for APTT; 10.5–14.0 s for PT; and 2.0–4.0 g/L for Fbg, 10.5–14.0 s for TT; and 0–700 µg/L FEU for D-dimer. Fbg and D-dimer results above the upper limit of the assay measurement range were excluded from statistical analyses.

Statistical analyses

Statistical analyses were performed using SPSS, version 16. Coagulation test results were not normally distributed and are therefore reported as median and interquartile range. To assess stability, the percentage changes (Result at storage time X – Result at baseline, divided by results at baseline, expressed as a % change) compared with the baseline results were calculated, and a clinically relevant difference was defined as a percentage change of >10% [9, 11].

Results following storage were compared with their respective baseline results using ANOVA to identify statistically significant differences. Least squares linear regression equations and Pearson correlation coefficients (*r*) between baseline results and results following 4, 8 and 24 h storage at RT and 4 °C were calculated. All statistical tests were two-tailed with *P*-values < 0.05 taken as significant.

RESULTS

Between- and within-batch imprecision

The manufacturer's product information for the assays includes claims that between- and within-batch

imprecision should be <15% and <5%, respectively, for PT, APTT, Fbg and TT, and between- and within-batch imprecision all should be <15% for D-dimer. In our laboratory, between- and within-batch imprecision of all analytes was all consistent with those claims. Detailed results are shown in Table 1.

Stability studies

Detailed data are shown in Table 2, and the mean percentage change trends of PT, APTT, TT, Fbg and D-dimer results over 24 h of storage at RT and 4 °C are shown in Figure 1. Both at RT and 4 °C, the mean percentage changes following 4, 8 and 24 h storage were <10% for PT, Fbg, TT and D-dimer, but the mean percentage changes following 24 h storage for APTT all exceeded 10% and thus exceeded the analytical within-batch imprecision and the clinical acceptability criterion we had specified. Changes for the PT, APTT, TT, Fbg and D-dimers results after 4, 8 and 24 h storage were all statistically significant at RT and 4 °C. Regression slopes between 0.9–1.10 and *r* > 0.975 were considered acceptable; all regression equation slopes were between 0.90 and 1.10, except the slope for APTT after 24 h storage at RT. All correlation coefficients were >0.975.

DISCUSSION

Acceptable time intervals between sample collection and measurement depend on the assay performed and on sample storage time and temperature [5]. We evaluated the effects of temperature and time variables on APTT, Fbg, D-dimers, PT and TT in our laboratory.

Previous studies of the relationship between storage time and coagulation test results have come to

Table 1. Within- and between-batch imprecision of Sysmex CA7000 assessed using samples giving results in the normal range and outside the normal range (abnormal)

	Within batch imprecision (%)		Between batch imprecision (%)	
	Normal	Abnormal	Normal	Abnormal
PT (S)	1.18	1.66	4.61	4.52
APPT (S)	0.53	1.57	4.32	3.86
TT (S)	1.22	1.55	4.51	–
Fbg (mg/L)	1.61	1.27	4.44	–
D-dimer(µg/L FEU)	1.80	1.98	4.91	4.55

–: no result. The abnormal value control product in our laboratory does not include TT and Fbg.

Table 2. Influence of RT and 4 °C on PT, APTT, Fbg, TT and D-dimer measurements

	Baseline	4 h	8 h	24 h
RT				
PT(S)†	12.25 (10.9–40.35)	11.8 (10.5–39.76)*	12.3 (10.61–39.615)*	12.3 (10.8–41.71)*
APTT(S)†	28.6 (20.61–81.5)	28.95 (21.01–82.16)*	30.15 (20.815–82.6)*	31.75 (22.02–87.89)*
TT(S)†	19.95 (14.945–62.19)	19.55 (14.55–60.85)*	20.15 (14.67–61.50)*	20.55 (15.08–63.76)*
Fbg(g/L)†	3.2 (1.6–7.36)	3.3 (1.5–6.99)*	3.1 (1.41–7.18)*	3.1 (1.31–7)*
D-dimer (µg/L FEU)†	680 (171.5–9980)	640 (161.5–8977.5)*	660 (171.5–9608)*	670 (171.50–9665.5)*
4 °C				
PT(S)†	13.5 (10.31–42.57)	13.2 (9.92–42.08)*	13.55 (10.42–42.69)*	13.9 (11.01–42.71)*
APTT(S)†	34.05 (19.86–81.04)	34.65 (19.90–82.)*	35.95 (20.00–86.20)*	37.5 (21.62–92.51)*
TT(S)†	19.20 (16.6–58.58)	18.8 (16.21–58)*	19.6 (16.42–58.99)*	19.8 (16.81–61.16)*
Fbg(g/L)†	3.45 (1.12–6.58)	3.40 (1.22–6.68)*	3.30 (1.12–6.19)*	3.30 (1.12–6.09)*
D-dimer (µg/L FEU)†	675 (170.5–8063.0)	670 (160.5–7841)*	630 (160.5–7406)*	670 (170.5–8043.5)*

* $P < 0.05$ comparison with Baseline results.

†Data are expressed as Median and 5th–95th percentiles.

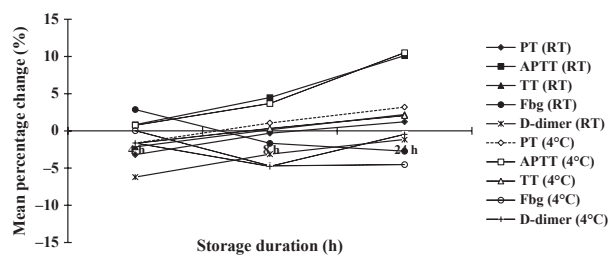


Figure 1. Mean percentage change (%) trend figure of RT and 4 °C on PT, APTT, Fbg, TT and D-dimer measurements following 4, 8 and 24 h storage.

various conclusions [6, 11, 12, 14]. At both RT and following refrigerated storage, Wang Xiao [6] and Rao [12] found that PT and APTT results were increased with duration of storage. Salvagno [13] found that PT and D-dimer results decreased initially before increasing but that APTT results increased gradually, while Fbg results were reduced markedly following 24 h storage. Kemkes-Matthes [14] found that PT, APTT and Fbg results after 8 and 24 h storage were all statistically different when compared with baseline results at RT, but found no differences for D-dimer. They further demonstrated that the differences exceeded the analytical quality specifications for desirable bias following 24 h storage.

The slopes and correlation coefficients of regression equations can be used to evaluate the effect of storage time on routine coagulation tests in whole blood [12, 14]. Salvagno [13] showed similar results to ours.

Wang B [15] demonstrated that after as little as 1 h, samples stored at RT may show significant differences compared with baseline results, although these differences would not have a significant impact on clinical diagnosis or treatment decisions. Thus, a statistically significant difference need not be the same as a clinically relevant difference. Zürcher [9] suggested that the imprecision of coagulation testing may have a greater impact on results than the changes due to storage time in citrated whole blood and proposed the calculation of the mean percentage change as a performance indicator in stability studies. In our study, to minimize analytical performance variability, we utilized a single assay kit batch for all analyses. We demonstrated good within and between-batch imprecision (both $<5\%$, as shown in Table 1).

Identification of a clinically relevant difference is difficult because there is no consensus defining clinical relevance. Most studies use percentage change [8, 9, 11, 14, 15]: Kemkes-Matthes [14] decided that sporadic changes exceeding 15% in more than 10% of samples defined clinical relevance. Adcock [8] defined a change by $>15\%$ as a clinically relevant difference. Wang B [15] defined a change $>10\%$ in PT, APTT or

TT, and >15% in Fbg as significant. There are no guidelines defining acceptable percentage pre-analytically for coagulation assays. We therefore used the same approach as Zürcher and defined a percentage change >10% from baseline results as a clinically relevant difference [9, 11]. Both at RT and 4 °C, the mean percentage changes following 24 h storage for D-dimer, Fbg, PT and TT were all <10%. For APTT, the change at 24 h exceeded 10%. Kemkes-Matthes [14] also decided that the acceptable bias strongly depends on the coagulation test concentration, so a wide range of normal and pathological samples should be assessed for change evaluation. We therefore selected a large enough patient group to ensure samples with low, normal and high coagulation test results.

Earlier studies vary in the acceptable time interval they identify. Salvagno [13] suggested that 6 h storage of uncentrifuged specimens at either RT or 4 °C may be acceptable. Wang Xiao [6] found PT and APTT could be analysed up to 8 and 6 h following collection at 4 °C and up to 4 h and 4 h at 25 °C. Wang B [15] found PT, Fbg and TT samples were acceptable up to 8 h following collection and APTT after up to 6 h at

RT. These acceptable time intervals were shorter than the acceptable time intervals recommended in the CLSI H21-A5 guidelines. However, other researchers have suggested that coagulation test samples can be stable for periods longer than currently acceptable time intervals recommended in the guidelines [8, 9, 11, 14]. Kemkes-Matthes [14] found that PT, APTT, Fbg, TT, AT and D-dimers could be reliably tested after storage for 8 h at RT, and storage time could easily be extended to 24 h for PT, TT and D-dimer. Rao [12] showed that PT and APTT could be accepted only up to 24 h and 18 h, respectively, when refrigerated or RT.

In conclusion, we found that samples for D-dimers, Fbg PT and TT were acceptable for analyses after up to 24 h storage duration and that APTT samples were acceptable up to 8 h storage at either room temperature or 4 °C. These storage time intervals meet our laboratory capability for coagulation testing.

The study was based on our study population; we did not study the PT and APTT results of patients on unfractionated heparin or coumarin-based oral anticoagulant therapy and therefore cannot comment on their stability.

REFERENCES

- Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. *Clin Chem Lab Med* 2006;44:358–65.
- Palmer RN, Gralnick HR. Inhibition of cold-promoted activation of the prothrombin time studies of new siliconized borosilicate collection tubes in normals and patients receiving warfarin. *Am J Clin Pathol* 1985;83:492–4.
- Palmer RN, Kessler CM, Gralnick HR. Warfarin anticoagulation: difficulties in interpretation of the prothrombin time. *Thromb Res* 1982;25:125–30.
- Palmer RN, Gralnick HR. Cold-induced contact surface activation of the prothrombin time in whole blood. *Blood* 1982;59:38–42.
- Adcock DM, Hoefner DM, Kottke-Marchant K, Marlar RA, Szamosi DL, Warunek DJ. Collection, transport, and processing of blood samples for testing plasma-based coagulation assays and molecular hemostasis assays. Approved guideline: fifth edition (H21-A5). CLSI 2008;28:1–33.
- Wang X, Ma J, Hao Z. Influence of storage time of PT and APTT at different temperature. *J Hebei Med Univ* 2002;23:108–9.
- Mohammed Saghir SA, Al-Hassan FM, Alsalahi OS, Abdul Manaf FS, Baqir HS. Optimization of the storage conditions for coagulation screening tests. *J Coll Physicians Surg Pak* 2012;22:294–7.
- Adcock D, Kressin D, Marlar RA. The effect of time and temperature variables on routine coagulation tests. *Blood Coagul Fibrinolysis* 1998;9:463–70.
- Zürcher M, Sulzer I, Barizzi G, Lammle B, Alberio L. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. *Thromb Haemost* 2008;99:416–26.
- van Geest-Daelderop 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.
- Sasse EA, Doumas BT, Gregory Miller W, D'Orazio P, Eckfeldt JH, Evans SA, Graham GA, Myers GL, Parsons PJ, Stanton NV. How to define and determine reference intervals in the clinical laboratory Approved Guideline-second Edition (C28-A2). CLSI 2000;20:1–59.
- Rao LV, Okorodudu AO, Petersen JR, Elghetany MT. Stability of prothrombin time and activated partial thromboplastin time tests under different storage conditions. *Clin Chim Acta* 2000;300:13–21.
- Salvagno GL, Lippi G, Montagnana M, Franchini M, Poli G, Guidi GC. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. *Int J Lab Hematol* 2009;31:462–7.
- Kemkes-Matthes B, Fischer R, Peetz D. Influence of 8 and 24-h storage of whole blood at ambient temperature on prothrombin time, activated partial thromboplastin time, fibrinogen, thrombin time, antithrombin and D-dimer. *Blood Coagul Fibrinolysis* 2011;22:215–20.
- Wang B, Guo W, Pan B. Influence of storage time at room temperature on routine coagulation tests. *Chin J Lab Med* 2011;34:595–7.