Stability of prothrombin time and activated partial thromboplastin time tests under different storage conditions

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Abstract

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are common laboratory tests that are useful in the diagnosis of coagulation disorders and monitoring anticoagulant therapy. Recent expansions in the outreach laboratory services at our institution prompted us to investigate the shipping limitations for some tests, including PT and aPTT. Although we followed NCCLS guidelines for the collection of blood specimens, we observed falsely elevated PT and aPTT values due to the different storage conditions. The objective of this study is to determine the effect of conditions and duration of storage on PT and aPTT tests using plasma and whole blood samples, respectively. For this study, 36 plasma samples with normal and prolonged PT and aPTT were exposed to different storage conditions. Blood was centrifuged immediately and plasma was stored at room temperature (RT), refrigerated at 4°C, or frozen at −20°C. The samples were analyzed at 0 h and repeated at 6, 12 and 24 h under various conditions. Although statistically significant differences were observed for plasma samples for normal PT tests after 12 h at refrigerated and frozen storage conditions, the differences would not change the clinical interpretation of the results. On the other hand, samples stored refrigerated or at RT showed significant differences for aPTT at 24 h. These differences would change clinical interpretation, especially for samples with normal or near normal aPTT times. Interestingly, aPTT was significantly higher for samples stored frozen when compared to refrigerated and RT conditions at 6 h. Similar patterns were also observed on ten whole blood samples with normal PT and aPTT values. In conclusion, either plasma or whole blood samples can be accepted for PT testing up to 24 h and for aPTT testing up...

to 12 h only, when transported either at RT or at 4°C. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are common laboratory tests used to investigate the extrinsic and intrinsic pathways of coagulation. These tests are performed diagnostically in any patient suspected of having a coagulopathy. PT is most often used to monitor the effects of Warfarin-based oral anti-coagulant therapy for patients who have had or at higher risk for heart valve replacement, deep vein thrombosis, pulmonary embolism, coronary thrombosis or other thromboembolic diseases. aPTT is usually ordered to monitor the effects of unfractionated heparin therapy. It can also be used to detect circulating anticoagulants such as Lupus anti-coagulant and anti factor VIII. In addition, it also detects congenital and acquired procoagulant deficiencies, except for Factor VII.

Recently, due to the tremendous growth in our outpatient clinical specimens, coagulation tests arrive in the laboratory from varying distances under variable storage and transportation modes. If NCCLS guidelines [1] are followed on the collection of blood specimens for coagulation testing, the allowable time interval (2 h, for samples maintained at room temperature (RT), 4 h if sample is stored at 2–4°C and 2 weeks at −20°C) between obtaining the specimen and performing the test place too many constraints on the laboratory. In addition, these guidelines do not stipulate whether samples should be kept as whole blood or plasma. In many instances, the long transportation times exceeded NCCLS guidelines, such that freezing plasma at −20°C was the only option. Although the samples were stored frozen, we were finding PT and aPTT values that did not correlate with clinical condition. A number of earlier studies suggested PT might be stable for periods longer than currently recommended in NCCLS guidelines [2–7]. In addition, it has been reported that aPTT samples are stable up to 8 h at either 4°C or RT, except for patients receiving unfractionated heparin therapy [6,7]. However, to our knowledge, no systematic studies have been published on the effects of time and storage conditions on both normal and elevated PT and aPTT samples.

In the present study, we investigated the stability of plasma samples with normal and elevated PT and aPTT values at 0, 6, 12 and 24 h, after storage at
RT, refrigerated and frozen conditions. We also evaluated the stability of whole blood samples with normal PT and aPTT values when stored up to 48 h at RT and refrigerated conditions.

2. Materials and methods

The stability of PT and aPTT tests under different storage conditions was studied in both separated plasma and whole blood.

For separated plasma study, blood specimens were collected from 36 incarcerated offenders of Texas prison system, on whom PT and aPTT had been routinely ordered over a 3-month period. These patients were not receiving any anticoagulant therapy at the time of collection. All blood specimens were drawn in 2.7-ml vacutainer tubes containing 0.129 mol/l (3.8%) sodium citrate (Becton Dickinson, Franklin Lakes, NJ, USA), giving a specimen mixture of 1 part of citrate and 9 parts of blood. Blood was centrifuged 10 min at 1500 rpm as soon as possible after collection (within 30 min). After centrifugation, plasma was removed and stored in three different aliquots and kept at RT, refrigerated (2±4°C) and frozen (−220°C) conditions. PT and aPTT were then analyzed on these samples after 0, 6, 12, and 24 h on a MLA-800 (Medical Laboratory Automation, Pleasantville, NY, USA) coagulation analyzer. The between-assay variability for PT and aPTT were analyzed at two levels. The coefficient of variation (CV) for PT was 1.3 and 2.1% at mean levels of 13.2 and 29.3 s. For aPTT, the CV was 1.6 and 1.4% at mean levels of 23.4 and 56.7 s, respectively.

For the whole blood study, blood specimens from ten healthy volunteers were collected in 2.7-ml vacutainer tubes containing 0.129 mol/l (3.8%) sodium citrate. These specimens were stored immediately at both refrigerated and RT conditions without centrifugation. PT and aPTT were analyzed on these whole blood specimens at 0, 12, 18, 24, 36 and 48 h intervals using an MDA-180 (Organon, Durham, NC, USA) coagulation analyzer. The between-assay precision for this analyzer is 0.65 and 0.80% at mean levels 12.3 and 26 s for PT, and 0.55 and 0.41% at mean levels 29 and 58.8 s for aPTT, respectively.

All measurements were performed in duplicate and the final value used was the mean of the duplicate results. International normalized ratios (INR) were calculated for the PTs performed on all tubes using a thromboplastin international sensitivity index (ISI) value of 1.94. In our institution, the normal range for PT is 11.5–13.5 s, and for aPTT is 21–34 s.

Differences between the different time intervals within same storage groups were evaluated by repeated measure analysis of variance and Fisher’s protected least significant difference (PLSD) method for multiple comparisons.
3. Results

3.1. Separated plasma

PT and aPTT were analyzed on all the 36 patients in this study and classified as normal ($n = 20$) and elevated ($n = 16$) depending on the initial result tested at 0 h.

For samples with normal PT, no overall statistically significant differences were observed, when samples stored at RT or refrigerated. However, statistically significant differences were observed when samples stored under frozen conditions ($P < 0.01$). When individual comparisons were made with time 0 ($12.69 \pm 0.13$; mean±SEM), no significant differences were observed at 6 h ($12.40 \pm 0.14$), 12 h ($12.54 \pm 0.16$) and 24 h ($12.88 \pm 0.10$) when samples were stored at RT (Fig. 1A). However, significant differences were observed between 0 h ($12.69 \pm 0.13$) and 24 h ($13.23 \pm 0.20$) when samples stored at refrigerated temperatures (Fig. 1A). In addition, significant differences were observed between 0 h ($12.69 \pm 0.13$) and 12 h ($13.57 \pm 0.27$), 24 h ($13.54 \pm 0.24$) when samples are stored frozen, thawed and tested at various time intervals (Fig. 1A).

For samples with elevated PT, no significant differences were observed when samples stores either at RT, refrigerated, and frozen conditions (Fig. 1B). Similar observations were noted in INR values also. The values at 0, 6, 12 and 24 h were $1.08 \pm 0.02$, $1.06 \pm 0.02$, $1.08 \pm 0.03$ and $1.12 \pm 0.02$ at RT, $1.08 \pm 0.02$, $1.13 \pm 0.02$, $1.18 \pm 0.02$ and $1.20 \pm 0.04$ at refrigerated conditions, and $1.08 \pm 0.02$, $1.19 \pm 0.03$, $1.26 \pm 0.05$ and $1.24 \pm 0.04$ for frozen storage condition.

On the other hand, samples with normal aPTT, showed overall statistically significant differences when samples stored at RT ($P < 0.001$), refrigerated ($P < 0.01$) or frozen ($P < 0.001$) conditions. When compared to the 0 level, the mean aPTT increased from $27.1 \pm 0.57$ (mean±SEM) to $31.9 \pm 0.72$ ($P < 0.001$) at RT, and to $29.8 \pm 0.62$ ($P < 0.01$) at refrigerated conditions at 24 h (Fig. 2A). However at frozen conditions the mean values increased to $31.24 \pm 0.94$ ($P < 0.01$) at 6 h, $32.55 \pm 1.3$ ($P < 0.01$) at 12 h and $32.62 \pm 1.2$ ($P < 0.01$) at 24 h, respectively (Fig. 2A).

Samples with elevated aPTT showed no overall statistically significant differences at RT, refrigerated, or frozen conditions. However, when compared to the 0 level ($48.9 \pm 2.7$; mean±SEM), significant elevations were observed when samples stored at frozen conditions at 6 h ($58.1 \pm 2.9$; $P < 0.05$), 12 h ($58.1 \pm 3.1$; $P < 0.05$) and 24 h ($59.2 \pm 3.2$; $P < 0.05$), respectively (Fig. 2B).

3.2. Whole blood

PT and aPTT were assayed in ten healthy volunteers in this study are classified as normal based on their initial result tested at 0 h. For PT, significant
Fig. 1. Effect of different storage conditions on normal (A) and prolonged (B), PT values. The samples are stored at room temperature (●), refrigerated at 4°C (○) and frozen at −20°C (▲). The values are represented as mean ± SEM (*, P < 0.05; **, P < 0.01; ***, P < 0.001), 0 h vs. 6, 12 and 24 h.
Fig. 2. Effect of different storage conditions on normal (A) and prolonged (B), aPTT values. The samples are at room temperature (●), refrigerated at 4°C (○) and frozen at -20°C (▲). The values are represented as mean±SEM (*, P < 0.05; **, P < 0.01; ***, P < 0.001), 0 h vs. 6, 12 and 24 h.
Table 1
Effect of different storage conditions on normal whole blood PT, INR and aPTT values\(^a\)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>PT RT (\pm) 0.11</th>
<th>PT 4(^{\circ})C (\pm) 0.11</th>
<th>PT-INR RT (\pm) 0.02</th>
<th>PT-INR 4(^{\circ})C (\pm) 0.02</th>
<th>aPTT RT (\pm) 0.02</th>
<th>aPTT 4(^{\circ})C (\pm) 0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.6 (\pm) 0.11</td>
<td>12.6 (\pm) 0.11</td>
<td>0.99 (\pm) 0.02</td>
<td>0.99 (\pm) 0.02</td>
<td>28.1 (\pm) 0.82</td>
<td>28.1 (\pm) 0.82</td>
</tr>
<tr>
<td>12</td>
<td>12.6 (\pm) 0.10</td>
<td>12.8 (\pm) 0.10</td>
<td>0.99 (\pm) 0.02</td>
<td>1.01 (\pm) 0.01</td>
<td>28.9 (\pm) 0.88</td>
<td>29.3 (\pm) 0.89</td>
</tr>
<tr>
<td>18</td>
<td>12.9 (\pm) 0.12</td>
<td>13.0 (\pm) 0.10</td>
<td>1.04 (\pm) 0.02</td>
<td>1.05 (\pm) 0.02</td>
<td>30.2 (\pm) 0.89</td>
<td>30.2 (\pm) 0.88</td>
</tr>
<tr>
<td>24</td>
<td>13.0 (\pm) 0.09*</td>
<td>13.0 (\pm) 0.13**</td>
<td>1.05 (\pm) 0.02</td>
<td>1.06 (\pm) 0.02*</td>
<td>30.8 (\pm) 0.93*</td>
<td>31.1 (\pm) 1.01**</td>
</tr>
<tr>
<td>36</td>
<td>13.2 (\pm) 0.16***</td>
<td>13.2 (\pm) 0.10**</td>
<td>1.08 (\pm) 0.03**</td>
<td>1.06 (\pm) 0.03**</td>
<td>31.5 (\pm) 0.98**</td>
<td>31.9 (\pm) 0.87**</td>
</tr>
<tr>
<td>48</td>
<td>13.6 (\pm) 0.17***</td>
<td>13.4 (\pm) 0.12***</td>
<td>1.16 (\pm) 0.03***</td>
<td>1.12 (\pm) 0.02***</td>
<td>31.6 (\pm) 0.90**</td>
<td>32.9 (\pm) 0.90**</td>
</tr>
</tbody>
</table>

\(^a\) Samples \((n = 10)\) were stored at RT and 4\(^{\circ}\)C, and tested at 0, 12, 18, 24, 36 and 48 h. The values are represented as mean \(\pm\) SEM; *, \(P < 0.05\); **, \(P < 0.01\); ***, \(P < 0.001\); 0 h vs. 6, 12, 18, 24, 36 and 48 h. Overall differences were observed when samples stored at RT \((P < 0.001)\) and refrigerated conditions \((P < 0.001)\). The individual mean \(\pm\) SEM values at times 0, 12, 18, 24, 36 and 48 h are represented in Table 1. When compared to time 0, statistically significant differences were observed at 24, 36 and 48 h in both PT and INR values (Table 1).

Similar observations were noted in aPTT values also \((P < 0.001)\) overall significance at both RT and refrigerated conditions (Table 1).

4. Discussion

A number of earlier studies suggested that PT and aPTT determinations might be stable for periods longer than currently recommended in NCCLS guidelines [2,4–6]. Koepke et al. [5] collected several tubes of blood from each of ten healthy subjects. Plasma was kept at RT for 24 h and aPTT were performed on plasma of one tube and repeated on other tubes at 2, 4, 6 and 24 h. They observed no change in PT and aPTT up to 6 h and 10–15% prolongation of aPTT at 24 h. Neofotistos et al. [8] also observed similar findings in their study which was carried only up to 8 h. Adcock et al. [6] evaluated the effects of time and temperature variables on PT and aPTT tests on different groups of patients that included healthy volunteers, hospitalized patients not receiving anticoagulants, patients receiving anticoagulants, and patients receiving unfractionated heparin therapy. Blood samples were either centrifuged immediately or whole blood stored at RT or on ice at 0, 4, 6, 8 and 24 h. Their data demonstrated that PT results are stable up to 24 h at both RT and on ice conditions. Whereas aPTT assays are stable up to 8 h except for patients receiving unfractionated heparin therapy. In the present study, we exposed plasma samples to three (RT, refrigerated and frozen) different types of storage conditions. PT and aPTT values at 0 h
were compared to 6, 12 and 24 h on the same samples. No significant differences were observed for both normal and elevated PT tests between 0 and 6, 12 and 24 h when samples were stored at RT. Although statistically significant differences were observed for plasma samples for normal PT tests after 12 h at refrigerated and frozen storage conditions, the differences would not change the clinical interpretation of the results. Similar observations were noted on whole blood normal PT samples without centrifugation, exposed to two different storage conditions (RT or refrigerated) and assayed at 0, 6, 12, 18, 24, 36 and 48 h.

aPTT tests showed significant differences between 0 and 24 h when plasma samples stored at either RT or refrigerated conditions. In addition, the aPTT tests on previously frozen and thawed samples showed significant elevations starting at 6 h. These differences would change the clinical interpretation especially for samples with normal or near normal aPTT. Whole blood aPTT specimens also showed significant elevations between 0 and 24, 36 and 48 h when stored either at RT or refrigerated conditions.

Overall, samples for aPTT testing are less stable than PT testing at both normal and prolonged values. From our study we have shown that samples for PT testing can be accepted up to 24 and probably 48 h for whole blood, when transported under either refrigerated or RT conditions. For aPTT testing, the separated plasma can be accepted up to 12 h when transported at either refrigerated or RT. The whole blood study for samples with normal aPTT values showed no differences for up to 18 h at either refrigerated or RT. However, more samples and prolonged aPTT values are needed to confirm this observation, as whole blood specimens in this study include only normal subjects. Surprisingly, in our hands, the frozen plasma specimens showed significant elevations at 6 h for aPTT and 12 h PT, and thus should not be used to transport samples. A number of factors affect the stability of coagulation factors present in blood samples after thawing [9]. In the present study, it is difficult to say why frozen and thawed specimens showed prolonged values compared to RT and refrigerated storage conditions. The problem with transporting specimens is that it is first assumed that they are rapidly frozen and that once frozen and maintained in this state until delivery to the laboratory. However, this may not have been the case for all specimens.

References


