Optimization of the Storage Conditions for Coagulation Screening Tests

Sultan Ayesh Mohammed Saghir¹, Faisal Muti Al-Hassan², Omar Saeed Alsalahi³, Faizatul Syima Abdul Mana³ and Huda Salman Baqir²

ABSTRACT

Objective: To determine the optimum storage temperature and time for prothrombin time and activated partial thromboplastin time at various intervals at both room temperature and refrigerator.

Study Design: Experimental study.

Place and Duration of Study: Advanced Medical and Dental Institute (AMDI), Laboratory at University Sains Malaysia (USM), from August 2009 to June 2010.

Methodology: After obtaining the consent, 33 blood samples were collected from AMDI staffs and students. Prothrombin time (PT) was measured at 0, 4, 8 and 24 hours (h). Partial thromboplastin time (APTT) was measured at 0, 2, 6 and 8 h both at room temperature (RT) and refrigerator.

Results: Thirty three subjects (14 males and 19 females, aged from 20 to 40 years) were involved. PT showed no significant differences at RT at 4 h, while significant differences after 8 h and 24 h at RT and after 4 h, 8 h and 24 h at refrigerator were observed. APTT showed no statistically significant differences at 2 h but showed significant differences at 6 h, 8 h at both RT and refrigerator.

Conclusion: Samples for PT testing can be accepted only up to 4 h when kept at RT while the samples cannot be accepted when kept at refrigerator for 4 h and above. APTT samples can be accepted up to 2 h only at RT or refrigerator.

Key words: Prothrombin time. Partial thromboplastin time. AMDI laboratory. Coagulation screening test. Storage conditions.

INTRODUCTION

Haemostasis is defined as an active process through which blood coagulation is started and completed in a rapid and steady managed style. Normal haemostasis occurs as a result of several managed processes to achieve two functions; it keeps the blood in a fluid state and causes a quick and restricted haemostatic block at the vascular injury site.

Haemostasis acts as a reconciler between procoagulant and anticoagulant forces. Procoagulant factors includes tissue factor (TF), serine proteases of the intrinsic and extrinsic pathways, cofactors, fibrinogen, plasminogen activator inhibitor and an activated charged cell surface membrane.

Primary haemostasis is a first response to endothelial damage such as normal endothelial turnover or tissue damage which results in the formation of a platelet block through interactions between platelets and vascular endothelium. On the other hand, secondary haemostasis is the formation of a stable fibrin clot over the already created platelet plug.

Secondary haemostasis occurs due to the consecutive activation of various coagulation factors that eventually produces thrombin at the site of vessel harm.

Haemostasis abnormality is investigated using prothrombin time (PT) and activated partial thromboplastin time (APTT). PT is a universal screening test that measures the efficiency of the extrinsic coagulation system. It contains factors I, II, V, VII, and X. APTT is also a universal test measures the competence of the intrinsic system and is used to investigate the abnormalities in factors I, II, V, VIII, IX, X, XI, and XII. It can also be used to find out circulating anticoagulants such as lupus anticoagulant and anti factor VIII.

According to the National Committee for Laboratory Standards (NCCLS) guidelines, coagulation tests should be done within 2 hour if kept at RT, 4 hour if sample is stored at 2-4°C and 2 weeks for samples preserved at -20°C. Several studies recommended that PT and APTT determinations may be constant for periods more than currently suggested in NCCLS guidelines.

On the light of the findings of previous studies and the delay in transporting samples for coagulation studies, this study aimed to determine the optimum storage temperature and time for PT, at 0 h, 4 h, 8 h and 24 h, and for APTT at 0 h, 2 h, 6 h and 8 h at both RT and refrigerator in Advanced Medical and Dental Institute (AMDI) laboratory, University Sains Malaysia (USM).
METHODOLOGY

Thirty-three healthy adult volunteers (20 - 40 years old) participated in this study from AMDI staffs and students. Individuals with chronic liver diseases or cardiovascular disorders, pregnant women, subjects under heparin therapy and age less than 20 years old were excluded. This study was approved by the Ethics and Research Committee of AMDI, USM. The participants had to sign the consent form before blood sample collection. Ten ml blood sample was drawn from each subject in vacutainer tubes containing 1 ml of 109 mmol/L sodium citrate (3.2%) giving a specimen mixture of 1 part of citrate and 9 parts of blood. All blood samples were centrifuged at 2500 rpm for 15 minutes. This was done within 30 minutes of collection. Approximately 4 - 5 ml of plasma was removed for each sample and divided into two parts, one stored in the refrigerator, and the other stored at room temperature (20.1 - 24.4°C).

The temperature of room and refrigerator was measured by using digital thermometer before doing any test. One-hundred µl of citrated plasma was incubated for 1 minute at 37°C. 200 µl of the PT warmed reagent (Stago) at 37°C was added to incubated plasma. Then the time from the plasma-reagent mixing to clot formation was measured in seconds using semi-automated machine (RAL). Control was measured at the same time with each sample.

One-hundred µl of citrated plasma was incubated for 3 minutes at 37°C with 100 µl of APTT warmed reagent (Stago), add 100 µl of warmed calcium chloride at 37°C. Then the time from addition of calcium chloride to clot formation was measured in seconds. Control was measured at the same time with each sample.

APTT and PT measurements were performed in duplicate and three or four times, for that samples which had difference more than one second, the final values were the mean of the results.

Sample size was calculated using Power and Sample Size Calculations software Version 2.1.31 (PS) program for paired test formula with 95% confidence interval. (σ = 4.0 represents standard deviation according to literature for paired test formula, δ = 2 desired Precision with Power 80% power is defined as 1- β, where β, the false negative rate). According to this formula, the sample size used in this study was 33 healthy subjects who were selected randomly from AMDI staff and students.

Data obtained was analyzed by using Statistical Package for Social Sciences (SPSS) version 16, by paired student t - test with confidence intervals 95% for comparison between the results at different time intervals with 0 h. Results were expressed as mean ± SD. APTT and PT measurements were performed in duplicate and three or four times for that samples which had difference more than one second. The final values were the mean of the results.

RESULTS

In this study, PT and APTT samples were analyzed and compared to the initial result tested at 0 h. The room temperature (RT) range was 20.1 - 24.4°C and the average was 22.9°C. The range temperature of the refrigerator was 3.4 - 4.7°C and the average was 4.3°C. PT results obtained at 0 h was compared with those obtained at 4, 8, and 24 hours at RT and refrigerator. All the results for 33 subjects were within the normal range (12.6 - 15.7 seconds). On the other hand, the measurements of APTT at 0 h were compared with measurements at 2, 6, and 8 hours at RT and refrigerator. All the measurements were within the normal range (28.9 - 38.1 seconds).

PT measurements at 0 h showed non-significant differences when compared with measurements at 4 h, while the differences were significant with measurements at 8 and 24 h at RT. On the other hand, all samples showed statistically significant differences when stored in refrigerator for 4 hours, 8 hours, and 24 hours as compared with measurements at time zero (Table I).

There was an increase in the PT results over time when samples were stored at RT with the maximum level (14.05 ± 0.12 seconds) at 24 h. On the other hand, when samples were stored in the refrigerator, the PT results obtained decreased over time and the minimum level was (13.1 ± 0.19 seconds) at 24 hours (Figure 1).

Table I: Comparison between the measurements at 0 h with the measurements at 4 h, 8 h and 24 h for PT at different storage conditions.

<table>
<thead>
<tr>
<th>Time</th>
<th>Room temperature</th>
<th>P-value</th>
<th>Refrigerator</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(h)</td>
<td>(22.9°C)</td>
<td></td>
<td>(4.3°C)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13.56 ± 0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13.61 ± 0.12**</td>
<td>0.016</td>
<td>13.27 ± 0.12***</td>
<td>0.001</td>
</tr>
<tr>
<td>8</td>
<td>13.79 ± 0.13***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>14.05 ± 0.12***</td>
<td>&lt; 0.001</td>
<td>13.18 ± 1.19**</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Samples (n = 33) were stored at room temperature & refrigerator and tested at 0, 4, 8 and 24 h. Values are represented as mean ± SD; paired sample t-test has been used. 0.05 considered significant. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 1: The effect of time and temperature on PT values at different storage conditions.
For APTT, comparisons between measurements at 0 hour and 2 hours showed non significant differences, when samples stored at RT or in refrigerator. However, there were statistically significant differences with measurements at 6 hours, and 8 hours at both RT and Refrigerator (Table II).

The means for APTT measurements were increased slightly when samples were stored for 2 hours at RT and refrigerator. Maximum level was observed at 8 hours at RT and refrigerator. Moderate increase was noted at 6 hours at both RT and refrigerator. The levels of APTT measurements were increased slightly over storage time for samples kept at RT and refrigerator (Figure 2).

### DISCUSSION

In coagulation studies, the diagnosis of coagulation disorders and monitoring of anticoagulant therapy usually depend on PT and APTT values. As a rule, coagulation tests should be carried out as soon as possible after collection of the blood samples. In this study, the findings are comparable to those reported by Koepke et al. and Neofotistos et al. who concluded that no changes were noted in PT and APTT up to 6 h, 10 - 15% prolongation of APTT after 24 h and no changes in PT and APTT up to 8 h respectively.

A previous study carried out by Adcok et al., on plasma samples reported different findings; they exposed plasma to three different types of storage conditions (RT, refrigerator and frozen). PT and APTT values at 0 h were compared with the measurements at 6, 12 and 24 h on the same samples. No significant differences were noted for both normal and elevated PT tests between 0 and 6, 12 and 24 h when samples were stored at RT, despite statistically significant differences were detected for plasma samples for normal PT tests after 12 h at refrigerator and frozen storage conditions, but the differences might not have altered the clinical analysis of the results.

In this study, an increase in the PT measurements was noted over time when samples were stored at RT, but a decrease in PT measurements were noted when samples were stored at refrigerator.

The variation in the PT values during the first few hours of storage were too small. Although statistically significant differences were observed for PT tests after 8 h and 24 h at both room temperature and refrigerator, the differences would not alter the clinical interpretation of the results.

In this study, APTT measurements showed an increase in the levels at both RT and refrigerator which were close to those reported by Tabata et al., who noted that APTT measurements were increased at 4°C while PT measurements were decreased at 4°C. Heil et al. demonstrated that APTT samples were stable up to 8 h at either RT or 4°C, except for those which were on unfractionated heparin therapy. This is slightly different from the present finding which demonstrated that storage time of APTT in refrigerator should not exceed 6 h.

These findings proved to be closer to the NCCSL guidelines, which recommend that the storage time of samples in refrigerator should not exceed 4 h, and they support the alternative hypothesis that there was a difference in PT and APTT at different storage temperature and time.

As mentioned earlier, different studies showed different results on the stability of PT and APTT at different storage conditions. Their observations are different from the findings of the present study. The differences may be due to several factors, which can affect the stability of coagulation factors such as the automated machines that were used in their studies compared to the semi-automated machine. In addition, different type of reagents, concentration of anticoagulant, and weather conditions may have affected the results.

A limitation of this study is that this study investigated only the effects of RT and refrigerator on PT up to 24 h and on APTT up to 8 h on healthy subjects. This study did not use specimen from patients on heparin or warfarin therapy. Thus, no statement can be made about the effects of these storage conditions on such samples.

### Table II: Comparison between the measurements at 0 h with the measurements at 2 h, 6 h and 8 h for APTT at different storage conditions.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Room temperature (22.9°C)</th>
<th>P-value</th>
<th>Refrigerator (4.3°C)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.04 ± 3.53</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>37.15 ± 0.52</td>
<td>0.313</td>
<td>37.30 ± 0.52</td>
<td>0.281</td>
</tr>
<tr>
<td>6</td>
<td>38.04 ± 0.50***</td>
<td>&lt; 0.001</td>
<td>37.90 ± 0.58**</td>
<td>0.003</td>
</tr>
<tr>
<td>8</td>
<td>38.38 ± 0.56***</td>
<td>&lt; 0.001</td>
<td>38.2 ± 0.58***</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Samples (n = 33) were stored at room temperature & refrigerator and tested at 0, 2, 6 and 8 h. Values are represented as mean ± SD; paired sample t-test has been used. 0.05 considered significant. * P < 0.05, **P < 0.01, ***P < 0.001.

Figure 2: The effect of time and temperature on APTT values at different storage conditions.
In the present study, more samples were needed to confirm the observations. In addition, no statement can be made about the effect of long-term storage on PT and APTT values and the effects of frozen storage conditions on the stability of PT and APTT.

CONCLUSION

PT Samples can be accepted only up to 4 h when kept at RT and cannot be accepted for 4 h and above when kept at refrigerator. APTT samples can be accepted up to 2 h only at RT or Refrigerator.

Acknowledgement: The Advanced Medical and Dental Institute, Research Incentive supported this study.

REFERENCES


