International Journal of Scientific Reports
*Geelani S et al. Int J Sci Rep. 2018 Jul;4(7):182-185*
http://www.sci-rep.com
pISSN 2454-2156 | eISSN 2454-2164

DOI: http://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20182729

**Original Research Article**

# Effect of storage time on prothrombin time and activated partial thromboplastin time: study at a tertiary care center in Kashmir valley

**Sajad Geelani[1], Gul Sajad Wani[1], Subuh Parvez Khan[2]*, Syed Mudasir Qadri[3], Javid Rasool[1], Syed Saulat Quadri[4], Fiza Parvez Khan[5]**

[1]Department of Clinical Haematology, [2]Department of Haematopathology, [3]Department of Internal Medicine, [5]Department of Pathology, Sher e Kashmir Institute of Medical Sciences, Srinagar, J&K, India
[4]Health Services, Kashmir, J&K, India

**Received:** 28 April 2018
**Revised:** 30 May 2018
**Accepted:** 01 June 2018

***Correspondence:**
Dr. Subuh Parvez Khan,
E-mail: khansubuh@gmail.com



## ABSTRACT

**Background:** Prothrombin time (PT) and activated partial thromboplastin time (APTT) are tests of haemostasis commonly employed in the evaluation of coagulopathies. Storage temperature and time interval between sample collection and testing can have a significant effect on results of coagulation tests. The aims of the study were investigate whether storage temperature and time influence the results of routine coagulation tests and whether any changes caused by delayed analysis results in a clinically relevant difference, as well as to establish our own acceptable storage temperature and time guidelines.
**Methods:** This study was conducted at Department of Clinical Haematology, in a tertiary care center in Kashmir valley. This study included 50 cases. Individuals with chronic liver diseases or cardiovascular disorders, subjects on anticoagulant therapy were excluded. 25 samples were observed at room temperature (RT) and 25 samples at 2-8°C. PT and APTT was measured at 0, 2, 4, 8, 16 and 24 hours both at RT and 2-8°C. Findings at 0 hr were compared to findings at 2,4, 8,16 and 24 hours in both the groups.
**Results:** In case of PT, reliable results were obtained up to 24 hrs either kept at RT or at 2 to 8°C and for APTT reliable results were obtained up to 4 hours kept at RT or at 2 to 8°C as there was no significant change during this period.
**Conclusions:** Coagulation test should be performed as soon as possible with PT being performed before 24 hours and APTT before 4 hours of collection of sample irrespective of whether the sample has been preserved at RT or in refrigerator.

**Keywords:** Prothrombin time, Activated partial thromboplastin time, Coagulation test

## INTRODUCTION

Haemostasis is a dynamic process in which blood coagulation is initiated and terminated in a rapid and tightly regulated fashion.[1]

Haemostatic mechanisms prevent the threat of fatal haemorrhage. Hemostatic plug is formed by the interaction between platelets and clotting factors. This plug staunches the flow of blood at the site of vascular injury. Investigations like prothrombin time (PT), activated partial thromboplastin time (APTT), Thrombin (TT), Fibrinogen Level, Fibrin disintegrated products (FDP), D-Dimers and Factor Levels are used to detect Haemostasis abnormality. Most common tests used in the evaluation of coagulopathies and monitoring of anticoagulant drug therapy and liver diseases are

Prothrombin time (PT) and activated partial thromboplastin time (APTT).[2]

If the PT/INR is prolonged but the APTT is not, the probable cause is related to Factor VII (FVII) while prolonged APTT is related to the intrinsic pathway– either factors VIII, IX, XI or the contact factors (Factor XII, Prekallikrein or High Molecular Weight Kininogen).

Pre-analytical and analytical variables including storage temperature and time interval between sample collection and testing can have a significant effect on results of coagulation tests.[3]

Pre-analytical variables including specimen collection, storage, temperature, transport, anticoagulant type, haematocrit, filling status of the sampling tube and centrifugation variable can potentially affect analysis results and by extension the medical care offered to patients.[4]

To minimize the negative effects of pre-analytical variables, the Clinical and Laboratory Standards Institute (CLSI) H21-A5 recommends that specimens should be tested within 24 h for PT and 4 h for APTT if stored at room temperature (25˚C). The new guideline have not mentioned about the storage timing at 2-8°C. However, several studies have observed varying stability results of PT and APTT with different plasma storage conditions.

***Aim of the study***

- Investigate whether storage temperature and time influence the results of routine coagulation tests.
- Whether any changes caused by delayed analysis results in a clinically relevant difference, as well as to establish our own acceptable storage temperature and time guidelines.

## METHODS

This study was conducted at Department of Clinical Haematology, in a tertiary center in Kashmir valley for a period of 2 months from from February 2018 to march 2018. This study included 50 cases. Individuals with chronic liver diseases or cardiovascular disorders, pregnant women, subjects on anticoagulant therapy were excluded. Blood samples were collected in a set of non vacuum plastic test tubes containing 3.2% trisodium citrate anticoagulant; ratio of the volume of blood: anticoagulant was 9:1. The samples were then mixed carefully and centrifuged at 3000 RPM (revolution per minute) for 10 minute to provide platelets poor plasma. This was done within 30 minutes of collection. Plasma from 25 Samples was separated and divided evenly into six tubes and the tubes were labelled as A, B, C, D, E and F and were kept at room temperature. The room temperature (RT) range was 20.1-22.4°C. Plasma from other 25 samples was labelled as 0, 1, 2, 3, 4, 5 and 6 were kept in refrigerator. The range temperature of the refrigerator was 3.0-5°C. PT and APTT of sample A and 0 were performed immediately and recorded. PT and APTT was done to Sample B and 1 after 2 hrs, sample C and 3 after 4 hrs, sample D and 4 after 8hrs, sample F and 5 after 16 hrs and sample F and 6 after 24 hrs. The measurement was done using fully automated machine Technoclone (Ceveron Alpha GmbH Austria). The values at A were compared with the values at B, C, D, E and F. Similarly the values at "0" were compared with the values at 1, 2, 3, 4, 5 and 6. Value of controls is shown in table 1: Statistical analysis was done using SPSS 20.0 software.

**Table 1: Control values for PT and APTT.**

| Value of PT low control | INR 0.90 |
|---|---|
| **Target:** | INR 0.87 Range ( 0.74 – 1.0) |
| **Value of PT high control:** | INR 2.97 |
| **Target :** | INR 2.87 Range ( 2.44 – 3.30) |
| **Value of APTT low control:** | 26.0 Sec |
| **Target :** | 27.8 Sec Range ( 23.6 – 32.0) |
| **Value of APTT high control:** | 68.0 Sec |
| **Target:** | 69.6 Sec Range ( 59.9 – 80.0) |
| **Value of PT normal control:** | 12.5 Sec |
| **Value of APTT normal control:** | 24.0 Sec |

## RESULTS

The total number of cases for the study was 50 including 30 males and 20 females, aged between 18-60 years. The samples obtained from the subjects was divided into two groups, each consists of 25 samples. Group 1 comprising of 25 samples was selected for study at room temperature and the remaining group comprising of remaining 25 was selected for study at 2-8°C. PT and APTT results obtained at 0 h were compared with those obtained at 2, 4, 8, 16 and 24 hours at RT. Findings at room temperature are shown in Table 2. Similarly PT and APTT results obtained at 0 h was compared with those obtained at 2, 4, 8,16 and 24 hours at 2 to 8°C.Findings obtained at 2 to 8°C are shown in Table 3.

This study showed that there was almost negligible difference in the test results from 0 to 2 hours and hence accurate results could be obtained up to 2 hours of storage either kept at room temperature or at 2 to 8°C. However reliable results for PT could be obtained up to 24Hrs either kept at room temp or at 2 to 8°C and for APTT reliable results could be obtained up to 4 hours kept at room temp or at 2 to 8°C as there was no significant change during this period (SD < 1.0).

**Table 2: Statistical analysis of samples at room temperature.**

| | Test | 0 hr | 2 hr | 4 hr | 8 hr | 16 hr | 24 hr |
|---|---|---|---|---|---|---|---|
| **Mean** | PT | 12.90 | 12.94 | 13.12 | 13.32 | 13.59 | 13.97 |
| | APTT | 28.92 | 29.12 | 30.19 | 31.18 | 32.66 | 34.30 |
| **Variance** | PT | 0.1541 | 0.1692 | 0.1746 | 0.2296 | 0.2370 | 0.2312 |
| | APTT | 10.821 | 12.802 | 22.750 | 25.512 | 27.973 | 34.178 |
| **SD (0 hr Vs)** | PT | | 0.282 | 0.155 | 0.212 | 0.487 | 0.756 |
| | APTT | | 0.141 | 0.898 | 1.598 | 2.644 | 3.80 |
| **Observation** | PT | 25 | 25 | 25 | 25 | 25 | 25 |
| | APTT | 25 | 25 | 25 | 25 | 25 | 25 |
| **P Value** | PT | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |
| | APTT | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |

**Table 3: Statistical analysis of samples at 2 to 8°C.**

| | Test | 0 hr | 2hr | 4 hr | 8 hr | 16 hr | 24 hr |
|---|---|---|---|---|---|---|---|
| **Mean** | PT | 13.0 | 13.08 | 13.29 | 13.56 | 13.87 | 14.37 |
| | APTT | 28.924 | 29.128 | 30.196 | 31.188 | 32.668 | 34.308 |
| **Variance** | PT | 0.2216 | 0.2222 | 0.3129 | 0.4007 | 0.4171 | 0.6321 |
| | APTT | 9.6119 | 10.3671 | 11.1220 | 11.5811 | 9.6522 | 9.4599 |
| **SD (0 hr Vs)** | PT | | 0.056 | 0.205 | 0.395 | 0.615 | 0.968 |
| | APTT | | 0.144 | 0.899 | 1.600 | 2.647 | 3.807 |
| **Observation** | PT | 25 | 25 | 25 | 25 | 25 | 25 |
| | APTT | 25 | 25 | 25 | 25 | 25 | 25 |
| **P Value** | PT | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |
| | APTT | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |

## DISCUSSION

This study included a total of 50 cases aged between 18 and 60 years. 30 males and 20 females were included. Samples from both males and females showed same degree of stability and hence didn't make any significant difference in the study. There was almost negligible difference in the test results from 0 to 2 hours in this study and hence accurate results can be obtained upto 2 hours of storage either kept at room temperature or at 2 to 8°C. However reliable results for PT can be obtained upto 24Hrs either kept at room temp or at 2 to 8°C and for APTT reliable results can be obtained upto 4 hours kept at room temp or at 2 to 8°C as there is no significant change during this period (SD <1.0). Feng et al conducted a study on seventy-two blood samples which were tested after storage for 0 (baseline), 2, 4, 6, 8, 12, and 24 h at 25°C (room temperature) and 4°C (refrigeration) in two centres.[5] This study demonstrated that samples for PT/INR could be safely stored for 24 h; and APTT for 12 h at 4°C and 8 h at 25°C. Zhao et al. [4] conducted the study where they found that a storage time interval up to 24 h for PT, D-dimers, FBG, and TT, and 8 h for APTT at either RT or 4 °C is acceptable. Oddoze et al conducted the study in which they concluded that PT is stable for 24 h and APTT is stable for 6 h regardless of the storage conditions.[6] In a similar study by Ikhuenbor et al prothrombin time (PT) & activated partial thromboplastin time (APTT) results were stable for upto 2 hours and remaining constant regardless of storage conditions.[7] Kemkes-Matthes et al In his study concluded that the acceptable storage time can be extended to 24 h for PT.[8] Adcock et al in his study demonstrated that PT results are stable for upto 24 h, remaining constant regardless of storage conditions.[9] APTT assay are stable for up to 8Hrs except for patients receiving unfractionated heparin therapy. Heil et al in his study found that stability in plasma, defined as the period during which there was a change of less than 10% from the initial value, was 24 hours for prothrombin time and 8h for activated partial thromboplastin time. These studies showed varying results on the stability of PT and APTT at different storage conditions.[10] However the differences between their study and the present study 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 fully automated machine used in this study. In addition, difference in sensitivity of reagents, concentration of anticoagulant, and weather conditions may have affected the results. A limitation of this study is that this study investigated the effect on PT and APTT at RT and at 2-8°C time upto 24 hours on healthy subjects. This study did not use specimen from patients on oral anticoagulant therapy. As such no statement can be made on such sample obtained from the patients on the anticoagulant therapy.

## CONCLUSION

Coagulation test should be performed as soon as possible. This study has shown that reliable results for PT can be obtained upto 24 hrs either kept at room temp or at 2 to 8°C and for APTT reliable results can be obtained upto 4 hours kept at room temp or at 2 to 8°C as there is no significant change during this period. This study showed results which are consistent with modified CLSI guide lines H21-A5, which recommends that specimens should be tested within 24 h for PT and 4 h for APTT if stored at room temperature (25˚C).

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the institutional ethics committee*

## REFERENCES

1. Nathan DG, Orkin SH, Ginsburg D, Look TA. Nathan and Oski's hematology of infancy and childhood. 6th ed. Philidalphia: Saunders; 2003.
2. Rao LV, Okorodudu AO, Petersen Jr. Elghetany M T. Stability of prothrombin time and activated partial thromboplastin time tests under different storage conditions. Clin Chem Acta. 2000:300:13-21.
3. 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.
4. Zhao Y, Lv G. 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. Int J Lab Hematol. 2013;35:566-70.
5. 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.
6. Oddoze C, Lombard E, Portugal H. Stability study of 81 analytes in human whole blood, in serum and in plasma. In the Canadian Society of Clinical chemistry. Elsevier Clinical Biochem. 2012;45:464 –9.
7. Ikhuenbor D, Aghedo F, Isah IZ., Iwueke, Oladigbolu RA, Egenti NB et al. Open J Blood Dis. 2010;6:79-88.
8. 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
9. Adcock D, Kressin D, Marlar RA. The effect of time and temperature variables routine coagulation tests. Coagul Fibrinolysis. 1998;9(6):463-70.
10. Heil W. Grunewald R, Amend M, Heins M. Influence of time and temperature on coagulation analytes in stored plasma. Clin Chem Lab Med. 1998;36(7):459-62.

**Cite this article as:** Geelani S, Wani GS, Khan SP, Qadri SM, Rasool J, Quadri SS, et al. Effect of storage time on prothrombin time and activated partial thromboplastin time: study at a tertiary care center in Kashmir valley. Int J Sci Rep 2018;4(7):182-5.