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Effect of Storage Time and Temperature on PT and APTT in Venous Samples

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Abstract

Objectives: To assess the impact of temperature and storage time on venous blood samples for PT and APTT analysis, to compare the mean percentage changes in PT and aPTT concerning standard reference values.

Methodology: This prospective experimental study included 200 consecutive samples from subjects visiting the outpatient department and admitted subjects not receiving anticoagulant therapy at the Department of Pathology, Benazir Bhutto Hospital, Rawalpindi. The study was conducted over six months following approval of the synopsis. PT and aPTT were measured at two temperatures: 4°C (refrigerator) and 25°C (room temperature) at 0, 6, and 24-hour intervals in venous blood processed as platelet-poor plasma. Data analysis was performed using SPSS-23.

Results: Of the 200 subjects, 107 were male, and 97 were female, with an average age of 47 ± 15 years (range: 20-79 years). A total of 156 (78%) samples were from outpatient visitors, and 44 (22%) from inpatients. The findings showed a time and temperature-dependent increase in both coagulation parameters. At 25°C, the average PT increased by about 7.05% and aPTT by 47.97% after 24 hours. In contrast, samples stored at 4°C had much larger increases; the average PT rose by 65.06% and aPTT by 91.68% after 24 hours. These findings suggest that storage at 4°C results in more evident percentage increases in PT and aPTT compared to 25°C, especially over 24 hours, showing greater instability at lower temperatures.

Conclusion: Both PT and aPTT should be measured within the first 6 hours after sample collection, as their reliability decreases over time. Storage temperature has a significant effect, with lower temperatures causing greater alterations in results over time.

Keywords: Prothrombin time (PT), activated partial thromboplastin time (aPTT), and coagulation.

Introduction

The coagulation system preserves hemostasis by maintaining a precise balance that prevents excessive bleeding and pathological clot formation.[1, 2] Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) are fundamental laboratory tests used to assess the coagulation cascade.[3, 4] These tests are indispensable for evaluating bleeding risks, monitoring anticoagulant therapy, and diagnosing coagulation disorders. The accuracy and reliability of PT and aPTT results can be significantly influenced by preanalytical factors, including sample collection techniques, storage conditions, and handling protocols. [5, 6] Storage temperature and duration are critical variables that can affect the stability of coagulation parameters in venous blood samples.[7]

Previous studies have demonstrated that improper storage conditions can lead to alterations in PT and aPTT values, potentially compromising clinical decision-making.[8-11] Research has indicated that the type of sampling vials, freezing and thawing methods, and storage temperatures can impact coagulation test results, although these effects are generally minimal when samples are stored at ultra-low temperatures (-70°C).[12, 13]

Zhao et al. further highlighted that coagulation samples stored at -80°C remain stable for extended periods, with PT values stable for up to one year and aPTT values for up to six months, compared to shorter stability periods at -20°C.[14] Another study found that citrated plasma samples retained stable PT, aPTT, and coagulation factor activities for up to three months at -24°C and up to 18 months at -74°C.[15] In contrast, samples stored at room temperature (24°C) showed no significant changes in PT and aPTT values for up to four hours, after which PT decreased by 3.7% and aPTT by 6.2% .[16] These findings highlight the importance of optimal storage conditions to ensure the integrity of coagulation test results

This study aims to investigate the impact of storage time and temperature on coagulation parameters, providing valuable insights into optimal sample handling practices.

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Methodology

This Cross-Sectional study was conducted at the Department of Pathology, Benazir Bhutto Hospital, Rawalpindi, over 6 months. A total of 200 samples calculated by WHO criteria, confidence interval; 95%, Population means: 11.7, Population standard deviation: 2.5, Absolute precision=1 were collected via non-probability, consecutive sampling.

Inclusion Criteria: Asymptomatic volunteers visiting outdoors for other concerns and aged between 20-80 years, hospitalized subjects not receiving anticoagulation therapy, i.e., oral anticoagulants or heparin

Exclusion Criteria: Subjects with a history of premorbid conditions like CLD (Chronic Liver Disease), vWD (von-Willebrand Disease), Hemophilia, DIC (Disseminated Intravascular Coagulation)

The study assessed the effect of storage temperature and time on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) using patient-derived platelet-poor plasma (PPP). PT and APTT were performed on samples at room temperature, 25°C, and refrigerator temperature, 4°C, at 0-, 6-, and 24-hour intervals. The patient's platelet-poor plasma was made by adding 9 volumes of test plasma and 1 volume of trisodium citrate, centrifuged at 2000g for 15minutes at 4°C. Four test tubes were placed in the water bath at 37°C. Hundred (100) ul of test plasma was added to the prewarmed tubes. After incubation for 2minutes, 200 µL of tissue thromboplastin (PT) reagent was added. After 6-8 seconds, clot formation was observed against shielded light, and time was noted. Its reference range is 10-14 seconds.

For the aPTT assay, which evaluates the intrinsic and common coagulation pathways (involving factors II, V, X, VIII, IX, and XI), kaolin and calcium chloride were prepared in separate pre-warmed test tubes. A 100 µL aliquot of test plasma was added to the kaolin-containing tube, followed by 100 µL of platelet substitute kaolin (aPTT reagent). The mixture was incubated with gentle mixing for 10 minutes. After incubation, 100 µL of calcium chloride was added and mixed thoroughly. Clot formation was monitored under shielded light, and the time was recorded using a stopwatch. The reference range for aPTT was established at 25-43 seconds.

Results

The cohort consisted of 107 male and 97 female subjects, with an average age of 47±15 years (range: 20–79 years). Female subjects had a mean age of 48±16 years, while male subjects had a mean age of 45±15 years. Of the total subjects, 156 (78%) were outdoor visitors for other purposes, and 44 (22%) were from the indoor department. (Table I)

Table I: Demographic characteristics of study participants.

Variable	n	%
Gender		
Male	107	53.5
Female	93	46.5
Age groups		
20-40	72	36
41-60	87	43.5
61-80	41	20.5
Department		
Indoor	44	22
Outdoor	156	78

The results showed that PT remains relatively stable at 25°C up to 6 hrs with a minimal increase of 2.03%. After 24 hours, the mean PT at 25°C rose to 13.21±1.65 (maximum: 27) with an increase of 22.41 %. Conversely, at 4°C, the PT value increased by 7.05% after 6 hrs

Table II: Percent change in Mean PT (seconds) over 24 hours at 25°C and 4°C.

Mean PT	Time interval(hrs)					P-value
	0 hrs	6 hrs	% increase	24 hrs	% increase	
25°C	12.34±0.56	12.59±0.84	2.03 %	13.21±1.65	22.41%	0.001
4°C	12.85±1.22	15.73±1.86	7.05 %	21.21±2.87	65.06%	0.001

Table III: Distribution of PT values by time and temperature. (n = 200)

	0 hr (25°C)	0 hr (4°C)	06hr (25°C)	6 hr (4°C)	24 hr(25°C)	24hr (4°C)
<12	8(4%)	32(16%)	8(4%)	0(0%)	6(3%)	0(0)
12-13	186(93%)	102(51)	183(91.5%)	16(8%)	162(81%)	0(0)
14-15	6(3%)	63(31.5%)	6(3.00%)	85(42.5)	24(12%)	0(0)
>15	0(8%)	3(1.5)	3(1.5%)	99(49.5%)	8(4%)	200(100)

escalating to 65.06% at 24 hours. Table II & III

The prolongation of aPTT over time was statistically significant ($p < 0.001$) at both storage conditions. The mean aPTT values increased up to 5.4% at 25°C at 06 hrs. After 24 hours, aPTT values rose further to 45.22 ± 12.59 s, showing an increase of 47.72%. However, in the case of storage at 4°C, there was a very significant increase of almost 48% even after six hours of storage, with an additional increase up to 91% after 24 hours of storage. Samples stored at 4°C showed a greater increase than those stored at room temperature, indicating reduced stability of intrinsic pathway factors at low temperature (Table IV & V).

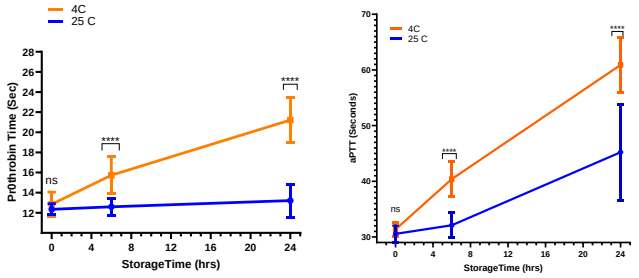

Figure 1a: Prothrombin time vs Storage Time

1b: Activated Partial Thromboplastin Time vs. Storage Time.

Data are presented as Mean± SD (n=200). Comparison between 25 °C and 4 °C at each time point was made using an independent sample t –test ****:p<0.0001,***:p<0.001,ns=not significant.*

Discussion

This study evaluated the impact of storage time and temperature on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) in venous blood samples processed as platelet-poor plasma. In this study, PT values showed progressive prolongation over time at both room (25°C) and refrigerator temperatures (4°C). A similar pattern was noted for aPTT, which increased to 60.09 ± 4.9 after 24 hours. The statistically significant changes ($p < 0.001$) indicate that both storage time and temperature substantially impact the stability of coagulation factors in plasma.

These findings are consistent with the observations of Gosselin et al., who reported that citrated plasma samples retain stable PT and aPTT results for up to four hours at room temperature, but beyond this period, significant prolongation occurs, particularly under refrigerated conditions.[17] The instability of PT and aPTT at 4°C observed in the present study may be attributed to cold-induced conformational alterations in temperature-sensitive factors such as VII, VIII, and IX.[18] Similarly, Zhao et al. found that plasma samples stored at –80°C remained stable for several months, while those stored at 4°C showed marked prolongation within 24 hours, particularly in APTT values.[5] The current results align with findings of Alesci et al., indicating that short-term refrigeration is unsuitable for coagulation testing when precise measurements are required.[19]

The differential effect of temperature between PT and aPTT can be explained by the greater instability of intrinsic pathway factors involved in aPTT, including factors VIII and IX.[20] Adcock et al. previously noted that aPTT is more sensitive to pre-analytical variations than PT, which is reflected in our data showing a 65% increase in PT at 4°C after 24 hours, compared to a 91% increase in aPTT[21]. This reinforces the role of aPTT as a sensitive indicator of plasma instability.

The mean value of PT at 25°C increased by 0.25 (2%) and at 4°C by 2.88 (22%) in a 6-hour interval. While in the study by Limin Feng and Ying Zhao, the value of PT decreased by 4.62 at 25°C and 2.08 at 4°C C after a 6-

Table IV: Percent change in Mean aPTT (seconds) over 24 hours at 25°C and 4°C.

Mean aPTT.		Time interval (hours)				P-value
	0 hours	6 hours	% increase	24 hours	% increase	
25°C	30.56±1.49	32.1±2.22	5.04	45.22±12.59	27.72	0.001
4°C	31.35±1.34	40.04±3.21	47.97	60.09±4.97	91.67	0.001

Table V: Distribution of aPTT values by time and temperature. (n = 200)

aPTT	0 hr (25°C)	0 hr (4°C)	06hr (25°C)	6 hr (4°C)	24 hr (25°C)	24hr (4°C)
≤30	115(57.5%)	66(33%)	56(28%)	0 (0)	23(11.5)	0(0)
31-35	84(4%)	133(65.5%)	125(62.5%)	16()	45(22.5)	0(0)
35-40	1(0.5%)	1(0.5%)	18(9)	91()	18(9)	0(0)
>40	0(0%)	0(0%)	1(0.5)	93()	114(57)	200(100)

hour interval.[22] In the study by Rogatko, the percentage of falls in PT value at room temperature after 6 hours was 3.7%.[16] In the study at Hangzhou, China, values of PT remain stable up to 6-hour intervals at room temperature and at 4°C. [5]

After 24 hours, the mean value of PT increased by 0.87(7%) at 25°C and 8.36 (65%) at 4°C. In other studies, the increase in mean PT value is 5.97 at 25°C and 6.58 at 4°C. The increase is similar to the results of our study.[22] In Zhao's study, the change in PT value is less than 1% at 25°C and 4°C. [5]

Feng et al observed an increase of 3.98 and 4.59 seconds after 6 hours at 25°C and 4°C, much more than our study.[22] In other studies, an increase of 0.35(1.2%) at 25°C and 0.6 (1.7%) at 4°C was observed.[5]

After 24 hours, an increase in value is significant, i.e., 14.66(46%) at room temperature (25°C) and 28.7(90%) at 4°C. While it is 20.4(71%) and 17.46(62%)[22] and 3.15(11%) and 3.45(10%)[5] respectively in other studies. it 45.22±12.59 (max 69) and 60.09±4.97 (max70) respectively.

The findings demonstrated that both parameters progressively prolonged with increasing storage duration, with greater instability observed in samples stored at 4°C compared to those kept at room temperature (25°C). The results also highlight that refrigeration promotes partial inactivation and cryoprecipitation of labile factors, especially factor VIII, leading to prolonged clotting times. These findings are in line with CLSI guidelines (H21-A5), which recommend that plasma samples for coagulation studies be tested within four hours of collection when stored at room temperature.[23] Any delay or inappropriate temperature control can lead to artifactual prolongation of coagulation times and potential clinical misinterpretation

Conclusion

The stability of the sample for Coagulation tests, i.e., PT and APTT, is up to 6 hours irrespective of the temperature it is stored. After that, the value increases significantly. However, guidelines say that the PT sample can be stored for up to 24 hours. The test should be performed without delay to get accurate results.

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