Original Research Article

Stability of Prothrombin time (PT) and activated partial thromboplastin time in frozen citrated plasma for 7 days

Jayesh Prabhakar Warade*

MD Biochemistry, Meenakshi Labs, Madurai, India
*Corresponding author email: **jdyajdo@gmail.com**

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Abstract

Background: Tests for hemostasis include prothrombin time (PT) and activated thromboplastin time (aPTT). Prothrombin time (PT) and activated partial thromboplastin time (APTT) are tests of hemostasis employed in the evaluation of coagulopathies and monitoring of anticoagulant drug therapy. These tests are supposed to be influences by many preanalytical factors.

Objective: In this study, we have evaluated the stability of aliquoted plasma of blood sample collected in sodium citrate tubes for prothrombin time and activated partial thromboplastin time stored at - 20 0C for a period of 7 days.

Material and Methods: 67 Patients samples collected for PT and APTT were stored for 7 days at – 20 ^{0}C in different aliquots. One aliquot is taken out daily and tested for PT and aPTT. Results were compared with initial tests results.

Results: It was found that there was no significant difference between initial test results and test results of the samples are stored at – 20^{0}C.

Conclusion: Samples which are collected for coagulation studies in sodium citrate preservative, if are centrifuged immediately and plasma is stored at – 20^{0}C, result generated for PT and aPTT do not show any statistically significant difference compared to samples which are immediately processed.

Key words

Plasma, Citrate, Prothrombin time, Coagulation, Stability.

Introduction

Tests for hemostasis include prothrombin time (PT) and activated thromboplastin time (aPTT). Prothrombin time (PT) and activated partial thromboplastin time (APTT) are tests of hemostasis employed in the evaluation of coagulopathies and monitoring of anticoagulant drug therapy. They measure the extrinsic and intrinsic arms of the coagulation cascade, respectively. These tests are supposed to be influences by many preanalytical factors. For coagulation study most important factors is sample collection with appropriate preservatives so that the integrity of coagulation factors can be maintained. Once the samples are collected for coagulation studies it may need to store or transport at another location in case immediate processing is not possible. During this period it must be seen that the stability of coagulation factors must be maintained so that results produced from examination processes are reliable and accurate.

Here in this study we have investigated the stability of hemostasis tests after storage of aliquot plasma collected using sodium citrate preservative.

Aim and Objective

In this study, we evaluated the stability of aliquoted plasma of blood sample collected in sodium citrate tubes for prothrombin time and activated partial thromboplastin time stored at - 20 ^{0}C for a period of 7 days.

Materials and methods

We collected 67 samples from patient who came for PT and aPTT testing with test request form from clinicians. The samples were collected in sodium citrate tubes and were immediately subjected to centrifugation for 15 minutes at 3500 rpm. The plasma was segregated and aliquot into 7 tubes for each sample with proper labeling. One aliquot from each patient was immediately process for PT and aPTT testing and remaining aliquots were stored at – 20 ^{0}C. Out of these each stored aliquots, each aliquot for each patient was taken out daily thawed and was processed for PT and aPTT testing. The results obtained by processing samples on day 1, day 3, day 5 and day 7 were compared with the results obtained by processing samples at 0 hr.

The data collected was analyzed using paired student ‘t’ test considering the statistical significant level at <0.05.

Results

The results obtained by processing samples at different time period on storage of the samples at - 20 ^{0}C are plotted in **Table - 1** and **Table - 2**.

Table - 1: Comparison of prothrombin time estimation at ‘0 hrs after collection and at 1 day, 3 day, 5 day and 7 day for samples stored at – 20 ^{0}C.

PT Estimation			
0 V/s 1 day	0 V/s 3 day	0 V/s 5 day	0 V/s 7 day
t is 1.536562. The value of p is .13804	t is 0.19676. The value of p is .84574.	t is 1.267555. The value of p is .21764	t is 1.932661. The value of p is .06568

Table - 2: Comparison of activated partial thromboplastin time estimation at ‘0 hrs after collection and at 1 day, 3 day, 5 day and 7 day for samples stored at – 20 ^{0}C.

APTT Estimation			
0 V/s 1 day	0 V/s 3 day	0 V/s 5 day	0 V/s 7 day
t is 5.015508. The value of p is .00004	t is 11.577552. The value of p is < .00001	t is 9.832898. The value of p is < .00001	t is 15.950425. The value of p is < .00001

Discussion

The samples were collected in sodium citrate tube followed by immediate centrifugation and confirmed for palate poor plasma (PPP). The samples were aliquot and on set were immediately tested for prothrombin time and activate partial thromboplastin time (aPTT). The samples were stored in – 20 ^{0}C for 7 days and were analyzed every day with each aliquot. The thawed samples were not again frozen and were discarded. The result obtained by testing these samples were compared with result of samples tested at 0 hour. The data was analyzed and it was found that there was significant difference between the results obtained by testing ample at 0 hour compared to the results obtained by testing the samples are 1 day, 3 day, 5 day and 7 day. It was found that the separated plasma for samples collected in sodium citrate tube remains stable at 7 days if properly stored at – 20 ^{0}C.

Many other researchers have also concluded similar findings in their studies with respect to short term or long term storage of citrated plasma for PT, aPTT testing.

Elshazali Widaa Ali, et al. did not observed any significant changes in PT, aPTT estimation for samples stored for 1 week, though they noted significant difference for sample storage more than 1 week at – 20 ^{0}C [1]. Woodhams B, et al. shown activated partial thromboplastin time, prothrombin time (%), thrombin time and other coagulation factors are stable for up to 3 months if frozen at - 24 degrees C or lower [2].

Parag Patil, et al. showed that the patient plasma samples for PT, INR, and APTT tests could be safely stored for up to 36 hours in the freezer. In the refrigerator, samples for PT and INR tests could be safely stored for up to 24 hours while the samples for APTT deteriorated at 12 hours [3]. Neofotistos D, et al. concluded that within an 8-hour period and with plasma on spun-down cells at room temperature, add-on tests for PT and APTT could be performed with results similar to what would be obtained from testing unstored sample [4].

Conclusions

In our study, we have concluded that, samples which are collected for coagulation studies in sodium citrate preservative, if are centrifuged immediately and plasma is stored at – 20 ^{0}C, result generated for PT and aPTT do not show any statistically significant difference compared to samples which are immediately processed. So, separated citrate plasma can be reliably used for requesting add on tests for PT, aPTT estimation or even for routine estimation if it is not possible to tests the samples immediately to any reason.

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