

## SAMPLE INTEGRITY ANALYSIS FOR PROTHROMBIN TIME AND INR AT DIFFERENT STORAGE CONDITIONS

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### ABSTRACT

According to the acceptable temperatures and storage time available in the literature for prothrombin time, samples need to be processed within four hours at laboratory temperature. But samples need to be stored if there is a need to retest on request and also for research purposes. With advancement in development of new reagents and equipment, whether the storage condition and time can be extended need to be evaluated. It is aimed to study the integrity of plasma samples for prothrombin time and INR at different storage conditions. A laboratory based comparative study was conducted to analyse the sample integrity of platelet poor plasma for prothrombin time and INR. The samples were processed by fully automated coagulation analyser at different storage conditions with regard to temperature and duration. There was no significant prolongation or shortening in the prothrombin time or INR with storage at room temperature upto 24 hours, refrigerated temperature upto 72 hours and freezing upto 3 months. Samples for prothrombin can be frozen for research purposes and refrigerated for repeat test.

**KEYWORDS :** Prothrombin Time, INR Storage for Coagulation

The Greek physician Hippocrates (460-ca. 377 B.C.), Celsus (25 B.C.- A.D 50), and Galen (A.D 130-200) observed that clotting occurs within minutes of blood draw usually as recently reviewed by Owen ( Owen and Charles, 2001) . The characterisation of coagulation factors and pathways have constituted an enormous undertaking, lasting over two hundred years since the measurement of certain plasma coagulation factors for the first time designated as prothrombin time. The prothrombin time measures coagulation factor of the extrinsic pathway Factor VII and factors of the common pathway namely factors I, II, V, and X. It is carried out by adding thromboplastin and an excess of calcium to the anticoagulated plasma and measuring the clotting time. PT is the most commonly used coagulation screening test in routine laboratories. The international normalized ratio (INR) was introduced by the world health organisation of human (WHO) in the early 1980's as a mean of standardizing PT results (PT) (Quick, 1935).

Pre analytical variables are very important in laboratory assessment of haemostatic and coagulation system. This includes specimen collection, anticoagulant type and concentration, hematocrit, transportation, centrifugation as well as assay method. Acceptable temperatures and storage time are available with different studies. But these tests sometimes need to be retested if requested. Samples need to be stored for research purposes also. With advancement in development of new reagents

and equipment, whether the storage condition and time can be extended need to be evaluated (Dacie et al., 2012). It is aimed to study the integrity of plasma samples for prothrombin time and INR at different storage conditions.

### MATERIALS AND METHODS

A randomized laboratory based comparative analysis done on samples from 20 adult patients with normal haematocrit. Institutional ethics committee approved the study and informed consent was obtained from patients. 20 patient samples were collected in evacuated tubes, containing buffered sodium citrate 0.109 M, 3.2%, made by BD, Franklin Lakes, NJ, USA. The fully automated coagulation analyser used for the prothrombin time is Sysmex CS-1600, Sysmex corporation Kobe, Japan, 2015. The clotting method depends upon the light transmittance (Sysmex, 2014). The reagent used was Tremblor S, Siemens, Germany- Human thromboplastin containing calcium with the ISI value 1.06. Two levels of commercial controls namely normal and abnormal are run twice a day and the PT assay and INR are involved in external quality assessment scheme. Platelet poor plasma (PPP) is prepared by centrifuging the sample for 15 minutes at 3500 rpm. The quality control had been done on the first sample received in the laboratory on the particular day by processing the plasma in Beckman Coulter LH 780 hematology analyser for platelet count. The platelet count of less than 10,000/cu.mm was considered desirable.

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Each sample was aliquoted in 13 containers with 100 µl each and were stored at different temperatures namely room temperature, refrigerated temperature of 4°C, freezing at -20°C and -70°C. The samples were processed for prothrombin time at the schedules mentioned

- i. Samples at room temperature- within 1 hour,8 hours, 12 hours, 24 hours.
- ii. Samples stored at 4°C- 24 hours, 48 hours, 72 hours.
- iii. Samples stored at -20°C- 1 week, 2 week, 3 week.
- iv. Samples stored at -70°C- 1 month, 2 months, 3 months.

Statistical analysis was done in R commander, software. Comparative studies between the values were done using student t-test. The prothrombin time and INR value done on the samples at room temperature within 1 hour is considered as the true value of the patient. The results obtained at different storages are compared with the true value with mean ± SD calculated. Any difference is considered significant, if the p value is less than 0.05 (p=0.05).

## RESULTS

The age of the patients ranged from 21 years to 80 years with a mean range of 47.4 years. The male : female ratio is 2:3. Hematocrit value of the samples varied from 29.9% to 42.3%. The PT and INR values at different storage conditions are shown in the table 1. The prothrombin time at

room temperature within 1 hour of testing ranged from 12.4 seconds to 56.4 seconds with the mean value of 19.47 ± 9.63 seconds. There were no differences noted in the PT values when compared to the PT value done at room temperature within 1 hour.

The INR at room temperature within 1 hour of testing ranging from 0.9 to 4.47 with a mean value of 1.5 ± 0.77. No difference was noticed in the INR values under storage conditions up to 1 month at -70°C. However, INR was low when done on samples stored at 2 months and at 3 months at -70°C. But this difference was statistically not significant. The INR done on samples stored at room temperature within 1 hour of testing ranged from 0.9 to 4.47 with a mean value of 1.5 ± 0.77. There was no difference noticed in the INR values under storage conditions up to 1 month at -70°C. However INR was low when done on samples stored at 2 and 3 months at -70°C. But this difference was statistically not significant (Table 1).

## DISCUSSION

There have been studies done on the sample integrity analysis for coagulation tests at different storage conditions. These studies have been done because repeat tests are requested often by the clinicians. There is a necessity to store samples for research purpose.

Storing the PPP at laboratory room temperature of 22°C to 24°C upto 24 hours had no effect on prothrombin

**Table 1 : Comparison of PT and INR Values Done at Different Storage Conditions**

Sl. No	Storage Conditions	PT Mean (±SD) sec	P-Value	INR Mean (±SD)	P-Value
1.	Room temperature 8hrs	18.7(11.1)	0.9971	1.5(0.9)	0.9971
2.	Room temperature 12hrs	17.9(5.3)	0.798	1.6(0.9)	0.798
3.	Room temperature 24hrs	20.7(6.7)	0.624	1.7(1.0)	0.624
4.	4°C-24hrs	20.8(11.4)	0.6902	1.6(0.9)	0.6902
5.	4°C-48hrs	19.9(9.3)	0.9469	1.5(0.7)	0.9469
6.	4°C-72hrs	19.9(8.2)	0.8808	1.5(0.7)	0.8808
7.	-20°C-1week	19.1(9.9)	0.9141	1.5(0.8)	0.9141
8.	-20°C-2week	20.0(9.1)	0.9772	1.5(0.7)	0.9772
9.	-20°C-3week	20.7(9.3)	0.701	1.6(0.7)	0.701
10.	-70°C-1month	19.4(10.2)	0.9769	1.5(0.8)	0.9769
11.	-70°C-2month	20.2(9.0)	0.7347	0.98(0.8)	0.7347
12.	70°C- 3month	20.9(9.3)	0.6469	0.91(0.7)	0.6921

time and INR. The same observation was noted by Matthes et.al when whole blood was stored up to 24 hours at room temperature(Matthes et al.,2011).But the study done in Nigeria showed significant difference with plasma stored at room temperature and at 4°C.However this study was carried out with semi-automated analyser.But he found difference at 8 hours and 24 hours(Sahgir et al.,2012).

Our study did not show any difference in PT and INR with refrigerated plasma samples at 4°C, up to 72 hours. It had been observed a 15% prolongation of PT with thawed fresh frozen plasma after storage at 4°C for 2 weeks(Lambo et al., 2007).Though this is done on thawed plasma, storage at 4°C did not show much effect on PT. It had been postulated earlier that PT became shorter at 4°C for the simple reason of activation of factor VII at lower temperature in siliconised tubes (Palmer and Gralnick, 1982, 1985).

Freezing the plasma at -20°C for up to 3 weeks and at -70°C up to 3 months did not show any difference in PT. The INR done at -70°C at 2 months and 3 months showed mild reduction; however, this difference is statistically not significant. The study done by NA Hassan et.al showed storage of plasma at -20°C at 1 week prolonged PT (Hassan et al., 2013). But a study done at Manchester by Poller L. et. al showed no significant prolongation of PT in coumarin plasma samples(Poller et al.,1999).The variations in PT differed from normal plasma and coumarin plasma as well with thromboplastin reagents like human based or rabbit based and also with the ISI value. The factors VII, V, X and II were stable on freezing for upto 3 months in a study. Storage at -74°C for up to 18 months showed only 10% variation, and 3% variation was noted due to freezing at -20°C and -70°C in another study(Alesci et al.,2009).

Though it is advisable to perform prothrombin time and INR within 4 hours at room temperature of laboratory, our study like few other studies have shown no significant variation with storage of platelet poor plasma at room temperature up to 24 hours, refrigeration up to 72 hours and 2 months respectively. However, the variables like coumarin and other drug effect and the thromboplastin reagent and the ISI value may influence the values to a certain extent.

## CONCLUSION

A laboratory based comparative study was conducted to analyse the sample integrity of PPP for prothrombin time and INR processed by fully automated coagulation analyser at different storage conditions. Randomly selected samples received in the laboratory within an hour period were separated for PPP, aliquoted and the PT and INR done within one hour of receipt of the samples. The values were used to compare the values obtained at 8 hours, 12 hours and 24 hours with aliquots stored at room temperature, 24 hours, 48 hours and 72 hours with aliquots stored at 4°C in the refrigerator, 1 week, 2 weeks and 3 weeks with aliquots freezed at -20°C and 1 month, 2 months and 3 months with aliquots freezed at -70°C. There was no significant prolongation or shortening in the prothrombin time or INR with storage at room temperature up to 24 hours, refrigerated temperature up to 72 hours, freezing up to 2 months. However, more number of samples has to be evaluated with longer duration of storage, so that maximum storage time can be derived.

Limitations of the study being the clinical variables like anticoagulant or other drug effect were not studied. Also factor VII activity if studied would have thrown more light on the study.

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