The Effect of Storage and Time of Blood Specimen Examination of Plasma Prothrombin Time and Activated Partial Thromboplastin Time Stabilities

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Abstract

This was an analytical study with cross sectional design. Collection of blood specimens was carried out by consecutive sampling during March-June 2019. Blood specimens in this study were healthy adults aged ≥21 years who underwent general medical check-up or blood donors who met the inclusion criteria and signed informed consent. A total of 71 samples were stored at room temperature and temperature 4-8 °C, then PT (prothrombin time) and aPTT (activated partial thromboplastin time) checks were carried out at 0, 4, 12, and 24 hours. Data were analyzed by using Kolmogorov-Smirnov Test, paired t test and Wilcoxon Signed Rank Test. There were differences in the results of PT in the room temperature specimens and 4-8 °C at 4 hours, 12 hours, and 24 hours with *p* value <0.001. This was also found in the aPTT parameter, but for specimens at 4-8 °C at the 24th hour examination the results of changes in aPTT were not statistically significant with *p* values 0.062. Different PT and aPTT tests at the two storage areas and at different examination times obtained different and statistically significant results with *p* value <0.001. Examination of hemostasis physiology requires special attention, where pre-analytic plays an important role which can influence the overall test results. The aspects of time and storage on the stability of PT and aPTT specimens are illustrated in this study where significant differences were obtained with *p* values <0.001. Examination and storage of coagulation study specimens in accordance with CLSI recommendations will provide accurate results and accurately describe the state of the specimen according to the patient's clinical condition.

Keywords: *activated partial thromboplastin time, examination time, preanalytic coagulation study, prothrombin time, hematology*

Introduction

Coagulation is a physiological routine test requested at a clinical laboratory to identify the occurrence of coagulation disorders and monitor anticoagulant therapy. In the last few decades, various ways have been developed to improve the quality of preanalytic, analytic and standardized coagulation tests. However, poor control and standardization of the preanalytic phase still needs to be improved as it disrupts the reliability of the results. Therefore, continuous efforts to identify and prevent preanalytic errors are needed[1,2].

Although the integrity of specimens is important for each laboratory test, coagulation testing that requires plasma specimens seems to be very sensitive to even small deviations from standard practice regarding anticoagulant concentrations, container materials, collection techniques, centrifugation, examination time and blood specimen storage. Coagulation physiological testing is a routine test carried out in laboratories in general, and in Dr. Soetomo Hospital, Surabaya, Indonesia[1,2].

Similar to reagents, specimens also decline over time, which leads to a number of false test result points.

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In most cases, laboratories can safely use manufacturer's or published study information to determine the stability of the specimen, but in some conditions the stability of the specimen is not available yet[3,4,5,6]. Because of this reason, research related to recommendations of storage and examination time on the stability of PPT and aPTT blood specimens in the testing of coagulation physiology in Dr. Soetomo Hospital laboratory is very important, considering that the results of an accurate, fast, and precise testing is closely related to the financing and quality of blood testing services at Dr. Soetomo Hospital, Surabaya, Indonesia.

Materials and Method

The study used analytical study with cross sectional design. Blood specimens in this study were obtained from healthy adults aged ≥21 years old who underwent general medical check-up or blood donor that met the inclusion criteria and was willing to take part in the study. The collection of blood specimens was carried out by consecutive sampling during March-June 2019. Blood specimens were calculated using equation:

$$\alpha = 5,\ 1\text{-}\beta = 90,\ \sigma = 5.8,\ \sigma^2 = 33.64,\ \mu_0 = 28.3,\ \mu_a = 30.7$$

$$n = \frac{\sigma^2 (z_{1-\alpha/2}+z_{1-\beta})^2}{(\mu_0-\mu_a)^2}$$

So that the value of blood specimen is 62. Blood specimen value was calculated using the hypothesis tests for a population mean (two-sided test). Patients were excluded from the analysis if they did not meet the inclusion criteria and did not sign informed consent.. Statistical tests were done using SPSS version 17.0. A total of 71 specimens were collected, then divided and stored at room temperature and 4 - 8 ° C. Afterwards, PT and aPTT at 0 hours, 4 hours, 12 hours, and 24 hours were assessed. Prior to the statistical test, normality test was performed using the Kolmogorov-Smirnov test. Normally distributed data was analyzed using paired t test while not normally distributed data was analyzed using the Wilcoxon Signed Rank test. Data was displayed as mean±standard deviation with p <0.05 significantly.

Results

A total of 71 specimens that met the inclusion criteria and signed the informed consent were obtained for this study.

Table 1. Difference Test of PT of Room Temperature and 4-8 °C Specimen Groups at 0 hour, 4 hour, 12 hour, and 24 hour.

Temperature	Storage Time	n	Mean ± Standard Deviation	Median (min-max)	Difference with 0 hour		
					Mean ± Standard Deviation	*p* Value	Details
	0 hours	71	10.05 ± 0.361	10 (95-11.2)			
Room Temperature	4 hours	71	9.85 ± 0.445	9.8 (9-11.6)	-0.199 ± 0.266	< 0.001	Significant
	12 hours	71	9.95 ± 0.351	9.9 (9.3-11)	-0.094 ± 0.150	< 0.001	Significant
	24 hours	71	10.22 ± 0.588	10.1 (9.3-12.5)	0.175 ± 0.572	0.045	Significant
4-8 °C	4 hours	71	10.10 ± 0.364	10.1 (9.5-11.3)	0.056 ± 0.198	0.001	Significant
	12 hours	71	10.13 ± 0.457	10.1 (9.2-11.4)	0.086 ± 0.259	0.007	Significant
	24 hours	71	10.41 ± 0.900	10.3 (7.9-15.8)	0.369 ± 0.897	< 0.001	Significant

Table 2. Difference Test of aPTT of Room Temperature and Specimen Group at 0 hour, 4 hour, 12 hour, and 24 hour.

Temperature	Storage Time	n	Mean ± Standard Deviation	Median (min-max)	Difference with 0 hour		
					Mean ± Standard Deviation	*p* Value	Details
	0 hour	71	29.01±2.160	29.1 (23.6-35.6)			
Room Temperature	4 hours	71	28.26±2.568	28.4 (23.5-35.0)	-0.751±2.054	0.090	Significant
	12 hours	71	30.60±2.120	30.2 (27.1-37.0)	1.587±1.222	< 0.001	Significant
	24 hours	71	31.48±2.417	31.3 (26.6-37.2)	2.475±2.089	< 0.001	Significant
4-8 °C	4 hours	71	29.38±1.956	29.2 (25.4-35.0)	0.370±0.872	< 0.001	Significant
	12 hours	71	29.61±2.645	29.4 (23.7-37.1)	0.597±2.067	0.008	Significant
	24 hours	71	29.53±2.141	29,6 (23.1-34.6)	0.518±2.304	0.062	Not Significant

Table 3. Difference Test Results of PT at 4th Hour, 12th Hour and 24th Hour at Room Temperature and at 4-8 °C.

Storage Time	Temperature	n	Mean ± Standard Deviation	Median (min – max)	Difference		
					Mean ± Standard Deviation	*p* Value	Details
4 hour	Room Temperature	71	9.85±0.445	9.8 (9-11.6)	0.255±0.347	< 0.001	Significant
	4-8 °C	71	10.10±0.364	10.1 (9.5-11.3)			
12 hours	Room Temperature	71	9.95±0.351	9.9 (9.3-11)	0.180±0.262	< 0.001	Significant
	4-8 °C	71	10.13±0.457	10.1 (9.2-11.4)			
24 hours	Room Temperature	71	10.22±0.588	10.1 (9.3-12.5)	0.194±0.817	0.001	Significant
	4-8 °C	71	10.41±0.900	10.3 (7.9-15.8)			

Table 4. Difference Test Results of aPTT at 4 Hours, 12 Hours and 24 Hours at Room Temperature and at 4-8 °C.

Storage Time	Temperature	n	Mean ± Standard Deviation	Median (min-max)	Difference		
					Mean ± Standard Deviation	p Value	Details
4 hours	Room Temperature	71	28.26±2.568	28.4 (23.5-35.0)	1.121±1.688	< 0.001	Significant
	4-8 °C	71	29.38 ± 1.956	29.2 (25.4-35.0)			
12 hours	Room Temperature	71	30.60 ± 2.120	30.2 (27.1-37.0)	-0.990±1.756	< 0.001	Significant
	4-8 °C	71	29.61 ± 2.645	29.4 (23.7-37.1)			
24 hours	Room Temperature	71	31.48 ± 2.417	31.3 (26.6-37.2)	-1.956±1.967	< 0.001	Significant
	4-8 °C	71	29.53 ± 2.141	29.6 (23.1-34.6)			

Discussion

Preanalytic conditions that can potentially cause errors in hemostasis testing include improper sampling or improper handling of specimens. In these circumstances, the test results do not accurately reflect the state of the specimen according to the clinical condition of the patient[7]. In this study, as many as 71 specimens that met the inclusion criteria were collected, then PT and aPTT examinations were carried out at two different storage locations, namely at room temperature and at 4-8 °C, each at 0 hours, 4 hours, 12 hours and 24 hours. Clinical and Laboratory Standards Institute (CLSI) recommends storing specimens for the testing of physiological hemostasis at room temperature or non-refrigerated temperature and examined in the shortest time possible, preferably 1 (one) hour after collection[8]. At the Clinical Pathology Laboratory of Dr. Soetomo Hospital, Surabaya, the testing of physiological hemostasis is a routine test. Several conditions that occur in the field after the collection of specimens, either from the inpatient room or from the outpatient specimen collection room, may cause a variety of time delays between the collection and examination of the specimen. This can certainly affect the results of the examination and it is feared that results cannot describe the condition of the specimen according to the clinical condition of the patient accurately. Therefore, the time between specimen collection and specimen examination needs to be considered.

Prior to the testing of physiological hemostasis, the specimen must be stored at room temperature to prevent deterioration of unstable clotting factors such as factor V and factor VIII, and specimens do not need to be delivered in refrigeration, but at temperature of 15-22 °C. Extreme temperatures must be avoided and delays in transportation can affect unstable factors (factor V, factor VIII), which may cause prolongation of freezing time due to loss of activity of these factors[9,10]. In accordance with CLSI guidelines, specimens must be assessed as soon as possible (ideally within 1 hour after collection) to maintain specimen integrity.[2]

According to the 71 specimens in this study, it can be generaly said that there was a difference in the results of PT and aPTT on specimens examined at room temperature and at 4-8 °C. The mean±standard deviation of PT obtained at room temperature showed values of (10.05±0.361) seconds at 0 hours, (9.85±0.445) seconds at 4 hours, (9.95±0.351) seconds at 12 hours and (10.22±0.588) seconds at 24 hours. The data obtained

revealed information on the tendency of prolonged PT from the examination of 4 hours until the examination of 24 hours. However, the median variation did not show great differences with PT minimum value (9.0 seconds) and maximum value (12.5 seconds), where numbers were still in the range of PT reference values, namely (9-12) seconds.

The mean±standard deviation of PT at temperature of 4-8 °C revealed results of (10.10 ± 0.364) seconds at 4 hours, (10.13 ± 0.457) seconds at 12 hours and (10.41 ± 0.900) seconds at 24 hours. According to this data, there was a tendency of prolonged PT from the time of specimen collection to the 24-hour examination. It is important to pay attention to the storage of specimens at 4-8 °C, where examination at 24 hours showed a wide range of minimum - maximum values of PT parameter, namely (7.9-15.8) seconds. This shows that the storage of specimens at temperature of 4-8 °C can affect factor VII, thus prolong PT value beyond the reference value.

Difference test results of aPTT parameter on the room temperature specimen group at 4 hours, 12 hours and 24 hours showed p value of p <0.05. It can be concluded that there were statistically significant differences between specimens assessed at 0 hours with specimens assessed at 4 hours, 12 hours and 24 hours. The mean±standard deviation obtained at room temperature showed values of (29.01±2.160) seconds at 0 hours, (28.26±2.568) seconds at 4 hours, (30.60±2.120) seconds at 12 hours and (31.48±2.417) seconds at 24 hours. According to the data it can be informed that there was a prolonging of results, however the median of aPTT was in a range that was not much different (28.4-31.3) seconds. In this study, difference test was done on the results of specimens that were immediately assessed (0 hours), with the results of specimens assessed at 4 hours, 12 hours and 24 hours. Mean±standard deviation was obtained with variations that were still in the range of the standard reference value or slightly prolonged. So, although results are statistically reported to be significantly different with p value of p <0.001, results need to be further clinically studied to know the patient's clinical condition and disease history.

Difference test results of aPTT at temperatures of 4-8 °C showed p value of p <0.001 at 4 and 12 hours, while at 24 hours, results showed p value of p = 0.0062. It can be concluded that statistically there was no significant difference in the results of aPTT at 24 hours. An interesting finding in the examination of aPTT at 4-8 °C and at 24 hours is that results showed very small differences, which were 0.23 seconds and 0.07 seconds, thus it can be concluded that there was no significant difference. This study is in accordance with the study conducted by Magnette *et al*. who concluded that physiological coagulation specimens are recommended to be stored at room temperature (15-25 °C) and the examination of specimens should be carried out <4 hours for whole blood specimens that are not centrifuged and and <1 hour for centrifuged samples. Further research with a wide variety of storage time and larger quantity of specimens may provide data or results with higher accuracy in the form of cut off time and optimum storage temperature for PT and aPTT examination[5].

Conclusion

Is sum, the examination of hemostasis physiology requires special attention, where pre-analytic processes play an important role and can influence the overall test results. The aspects of time and storage on the stability of PT and aPTT specimens are illustrated in this study where a significant difference with p value of p <0.001 was obtained. Testing and storage of physiologic hemostasis specimens in accordance with CLSI recommendations will provide accurate results and accurately describe the state of the specimen according to the clinical condition of the patient.

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