

Assessment of Stability of Activated Partial Thromboplastin Time, D-Dimer, Fibrinogen, and Thrombin Time Under Different Storage Conditions in Human Plasma

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01. Introduction

The laboratory testing process is divided into three phases: pre-analytical, analytical, and post-analytical. Various pre-analytical factors can affect the accuracy of coagulation test results. These factors include the sampling technique, order of draw, anticoagulant type and concentration, hematocrit levels, tube filling, transportation, centrifugation, temperature, the time between collection and testing, as well as storage and assay methods [1]. According to the Clinical and Laboratory Standards Institute (CLSI) document H21-A5, specimens for activated partial thromboplastin time (APTT), D-dimer (DD), fibrinogen (FBG), and thrombin time (TT) tests should be analyzed within 4 hours if stored at room temperature (20-25°C). However, if frozen, these specimens remain stable for 6 months for APTT, 24 months for DD, 18 months for FBG, and 3 months for TT [2].

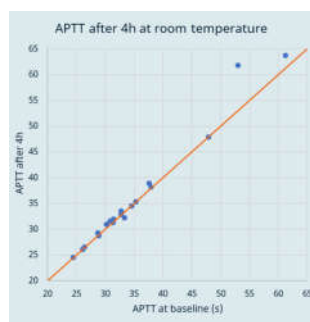
02. Aims and objectives

This study aimed to assess the effects of storage time and temperature on the stability of common coagulation tests in human plasma:

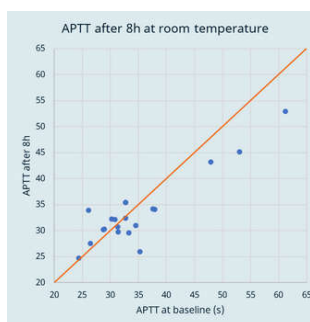
- activated partial thromboplastin time (APTT),
- D-dimer (DD),
- fibrinogen (FBG),
- thrombin time (TT).

04. Results

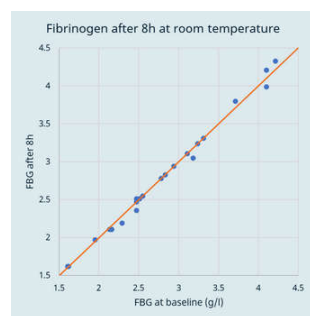
Of the 80 samples, 79 were included in the final analysis. One sample was excluded from the study due to insufficient volume of plasma. At room temperature, DD and FBG tests were stable for up to 8 hours, while APTT samples remained stable for only 4 hours, with instability observed at 6 and 8 hours (average changes of 9.0% and 9.3%, respectively). In frozen storage, samples for DD, FBG, and TT tests remained stable for 24 hours, with changes of less than 5% from baseline. However, APTT samples showed an average change of 10.6%, indicating instability.



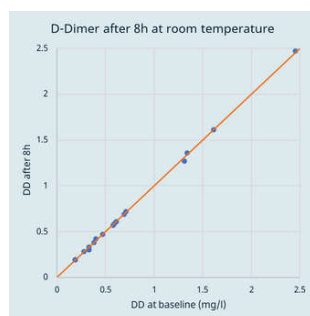
$r(19)=0.989$; $p<0.001$



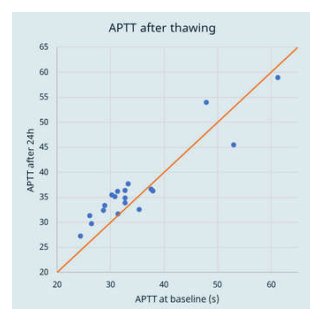
$r(19)=0.906$; $p<0.001$



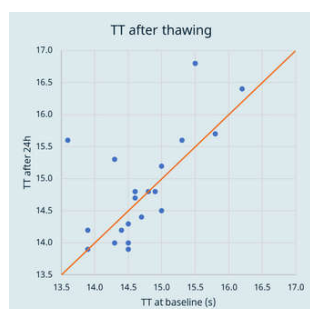
$r(20)=0.997$; $p<0.001$



$r(20)=1.000$; $p<0.001$



$r(19)=0.932$; $p<0.001$



$r(20)=0.651$; $p<0.001$

Comparison of baselines values with values after 4, 8, and 24 hours of storage. APTT: activated partial thromboplastin time; DD: D-dimer; FBG: fibrinogen; TT: thrombin time; h: hours

03. Methodology

Whole blood samples were collected from 80 patients using standard venipuncture techniques under aseptic conditions, into 2.7 mL BD Vacutainer vials containing 3.2% buffered sodium citrate. Samples that were inadequately filled, hemolyzed, icteric, or lipemic were excluded from the study.

Within two hours of collection, the samples were centrifuged at 2,000 g for 15 minutes to obtain platelet-poor plasma. Initial analyses of APTT, DD, FBG, and TT were conducted immediately on 20 samples for each test, with these results serving as baseline values. The remaining plasma for APTT, DD, and FBG was divided into two portions:

- one portion was stored at room temperature (20-25°C) and tested at 4-, 6-, and 8-hours post-collection;
- while the other portion was frozen at -20°C and tested at 24 hours post-collection.

The TT samples were only tested after freezing. After thawing, the analyses were completed within 30 minutes. All tests were performed using the Sysmex CN3000 automated coagulation analyzer, and a single batch of assay kits was used for all analyses to minimize variability.

Statistical analysis

All data were summarized and analyzed using Microsoft Excel and SPSS software, version 29. Relative change of result values from baseline was calculated. A change of more than ±5% from the baseline value was considered clinically significant. The values for fresh and stored samples were further evaluated using a Pearson correlation, with p-values less than 0.05 indicating statistically significant correlation.

05. Comparison with other sources

Source	Condition	APTT	DD	FBG	TT
Feng et al. [1]	RT	<8h	-	<8h	<8h
	Freezer	-	-	-	-
Kemkes-Matthes et al. [3]	RT	<8h	<24h	<8h	<24h
	Freezer	-	-	-	-
Zhao et al. [4]	RT	<8h	<24h	<24h	<24h
	Freezer	-	-	-	-
Geelani et al. [5]	RT	<4h	-	-	-
	Freezer	-	-	-	-
Patil et al. [6]	RT	<12h	-	-	-
	Freezer	<36h	-	-	-
CLSI H21-A5 [2]	RT	<4h	<4h	<4h	<4h
	Freezer	<6m	<24m	<18m	<3m
This research	RT	<4h	<8h	<8h	-
	Freezer	0	<24h	<24h	<24h

CLSI: Clinical and Laboratory Standards Institute; RT: room temperature; APTT: activated partial thromboplastin time; DD: D-dimer; FBG: fibrinogen; TT: thrombin time; h: hours; m: months

06. Conclusion

At room temperature, DD and FBG samples remain stable for up to 8 hours, while APTT samples are only stable for 4 hours. Plasma samples for D-dimer (DD), fibrinogen (FBG), and thrombin time (TT) tests can be stored in the freezer for up to 24 hours without significant change. However, in this study, activated partial thromboplastin time (APTT) samples were found to be unstable under these conditions.

References

- 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; 4:3868. doi:10.1038/srep03868.
- CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma- Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition. CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008; 15, 23.
- 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(3):215-220. doi:10.1097/MBC.0b013e328343b97e.
- 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(5):566-570. doi:10.1111/jlth.12090.
- Geelani S, Wani GS, Khan SP, Qadri SM, Rasool J, Quadri SS, Khan FP. Effect of storage time on prothrombin time and activated partial thromboplastin time: A study at a tertiary care center in Kashmir valley. *Int J Sci Rep.* 2018;4(5):182-185. doi:10.18203/issn.2454-2156.
- Patil P, Sehgal T, Goswami P, Gaur M, Khan M, Pandey S, Datta SK. Assessment of stability of prothrombin time, international normalized ratio, and activated partial thromboplastin time under different storage conditions in human plasma. *Cureus.* 2022;14(1) doi:10.7759/cureus.21268.