Study of Stability of Various Biochemical Analytes in Sample Stored at Different Storage Conditions

Tomcy A. T.[1], Bineesh.C.P[2,3,*], Anush N[3,4], Pranav Kumar Prabhakar[3], Sajith Chandran[5]

A. Laboratory trainee, Department of Biochemistry, Malabar Cancer Center, Thalassery, Kannur Kerala India
B. School of Applied Medical Science, Lovely Professional University, Punjab India- 144411
C. Department of Medical Biochemistry, Co-Operative Institute of Health Sciences, Thalassery, Kannur Kerala India 670105
D. Vocational Teacher in MLT, Sree Narayana Vocational Higher secondary School, Sreekanteswaram, Cherthala, Kerala, India-688526
E. Laboratory Head, Cooperative Hospital, Thalassery, Kannur Kerala India

ADDRESS FOR CORRESPONDENCE:

BINEESH.C P
Assistant Professor
Department of Medical Biochemistry,
Co-Operative Institute Of Health Sciences, Thalassery, Kannur Kerala 670105
EMAIL: bineeshcp87@gmail.com

ABSTRACT

A common problem in clinical laboratory is maintaining stability of biochemical analytes during sample storage. We usually keep samples in a refrigerator or at RT for short durations. Or in a freezer for long durations. Hence time and temperature are two important parameters that affect analysis result. The test result should be accurate and precise for better diagnosis, prognosis and monitoring of disease. The aim of the study was to determine whether the stability of biochemical analytes like glucose, AST, and creatinine is affected by different storage conditions of temperature and time. A set of 30 pooled plasma sample obtained from lithium-heparin tube, each one is aliquoted into 10 different tubes, one from each set is taken for baseline measurement (T1d). The remaining 9 is divided into 3 groups: Group1 Frozen (0 to -8 ℃) Group2 Refrigerated (2 to 8 ℃) & Group3 Room temperature (22 to 27 ℃).30 aliquotes from each group is measured for glucose, AST & creatinine on 4th (T4d), 8th (T8d) & 15th (T15d) day respectively. The mean change were compared with baseline measurement (T1d). The study was performed from 2nd May to 24th July 2019 at Thalassery cooperative hospital. The result obtained shows in sample kept at RT the concentration of glucose keep decreasing whereas creatinine increases and AST shows a variable concentration as the time progress. Statistically significant variation observed in sample kept at room temperature. In short stability of T1d>T4d>T8d>T15d & in group, stability of Group1>Group2>Group3. It was concluded that the parameters are most stable in the immediately separated plasma sample following 15days of storage at freezer, variations shown only in refrigerator and RT sample with the progress of time. It suggests time and temperature mediated mechanism are cause of these changes.

KEY WORDS: Plasma, Glucose, AST, Creatinine, stability, time, temperature.

1.INTRODUCTION:

The aim of the clinical laboratory test is to determine the true value of a diagnostically relevant analyte in our body fluid. Hence, stability becomes a critical consideration here, there are conditions like, breakdown of the equipment, physician order for add on testing, specimen received through mail, etc....In all these conditions the specimen may be exposed to longer time delays and varying temperature conditions. Also, in a laboratory the sample are deep frozen below 0°C for long time period storage or kept in refrigerator at 2 to 8°C for short durations, and kept in room temperature at 23 to 27 °C before examination. Hence time and temperature are two important factors that affect test results. For an accurate and precise result maintaining the stability of biochemical analytes during sample storage is necessary.

Previous studies in serum[1,2] performed in unseparated serum sample[3] also in serum separated from cells with gel barrier[4,5] and in serum immediately separated from cells[6,7]. Most of the studies were conducted with animal sample[8].

Present study determine the stability of plasma glucose, aspartate aminotransferase (AST), and creatinine kept at different storage conditions of freezer (0 to -8 DC) refrigerator (2 to 8 DC) and room temperature (22 to 27 DC) for 1st,4th,8th, and 15th day.

2.MATERIALS AND METHODS:

STUDY DESIGN

This hospital-based study included 70 to 80 random samples from outpatients being treated at thalassery cooperative hospital from 2nd May to 24th July 2019. The samples collected from each patient were for physician ordered laboratory testing; no additional blood was taken from the subjects. The Institutional Ethical Committee approved the study.

SAMPLE COLLECTION AND ANALYSIS

Due to limited sample volume available from days left over, plasma sample collected in lithium heparin tube of 4 to 5 patients were pooled and taken as a single sample in such a way 30 different samples were prepared. Each one is aliquoted into 10 different eppendorf tubes, it is then divided into 3 groups; 9 tubes in each group. Group 1— frozen (0 to -8 °C), Group 2— refrigerated (2 to 8 °C), Group 3— room temperature (22 to 27 °C)

The remaining 30 aliquotes were used for baseline measurements (T1d), 30 aliquotes from each group were alanysed on 4th (T4d), 8th (T8d), and 15th (T15d) day in siemens dimension rxl max which works on the principles of spectrophotometer. Then the mean change were compared with the baseline values (T1d).

Table 1: Methods used for the analysis of biochemical parameters

ANALYTE	UNIT	METHOD
GLUCOSE	Mg/dl	HK-G6PDH METHOD
AST(Aspartate aminotransferase)	U/L	IFCC method
CREATININE	Mg/dl	Modified jaffe reation

ANALYSIS: To determine time and temperature dependent changes in the plasma, the mean value from all the samples was calculated for each analyte at each point of study. Statistically significant changes were determined for each analyte by repeated measures ANOVA with a Greenhouse-Geisser correction method in SPSS Version 20.

3.RESULTS:

The tables below shows the mean and standard deviations of Glucose, AST, and creatinine.

Table 2: Mean and standard deviations of glucose

MEAN ± SD	FREEZER	REFRIGERATOR	ROOM TEMPERATURE
T1d			146.97±18.25
T4d	146.2±18.53	145.9±18.2	137.26±18.7
T8d	146.6±18.57	145.5±18.95	101.3±20.85
T15d	146.7±18.30	145.2±18.21	23.8±11.89

Table 3: Mean and standard deviations of AST

MEAN ±SD	FREEZER	REFRIGERATOR	ROOM TEMPERATURE
T1d			46.63±10.60
T4d	46.2±10.43	45.13±10.34	36±8.22
T8d	47.63±11.1	47±11.13	59.43±12.8
T15d	45.93±10.61	45.83±10.05	38.16±12.30

Table 4: Mean and standard deviations of creatinine

MEAN ±SD	FREEZER	REFRIGERATOR	ROOM TEMPERATURE
T1d			1.35±0.39
T4d	1.35±0.381	1.35±0.382	1.54±0.39
T8d	1.35±0.378	1.35±0.378	1.93±0.536
T15d	1.41±0.488	1.36±0.381	2.352±0.557

Table 5: statistical analysis of time and temperature dependent change in biochemical analytes

	GLUCOSE			AST			CREATININE		
	RT	R	F	RT	R	F	RT	R	F
F value	(1.61, 46.60) = 966.756	(2.286, 66.298) = 6.994	(3, 71) = 1.164	(2.216, 64.263) = 65.17	(2.142, 62.117) = 8.161	(2.124, 61.592) = 7.074	(2.129, 61.734) = 138.858	(2.49, 72.28) = 2.08	(3, 32) = 1.38
p value	< 0.001	>0.05	=0.328	<0.001	>0.05	>0.05	<0.001	=0.121	=0.253
Statistical significance	Sig	Not sig	Not sig	Sig	Not sig	Not sig	Sig	Not sig	Not sig

KEY; RT —room temperature, R —refrigerator, F-freezer, Sig- significant

The mean and standard deviations of analysis results for the biochemical analytes glucose, AST and creatinine measured in plasma samples under different storage conditions are shown in the tables above (table 2, 3 & 4). The analytes were most stable in sample stored at 0 to -8 °C (freezer), fairly stable in sample stored at 2 to 8 °C (refrigerator) throughout the study period. Compared with the baseline values (T1d) statistically significant variations were observed only in samples stored at 22 to 27 °C (room temperature) (Table 5) (Fig 1, 2 & 3). However, there was a statistically significant increase observed in creatinine concentration, with a reduction in glucose level in samples kept at room temperature. AST did not show any increasing or decreasing trend throughout the study period, rather keep fluctuating.
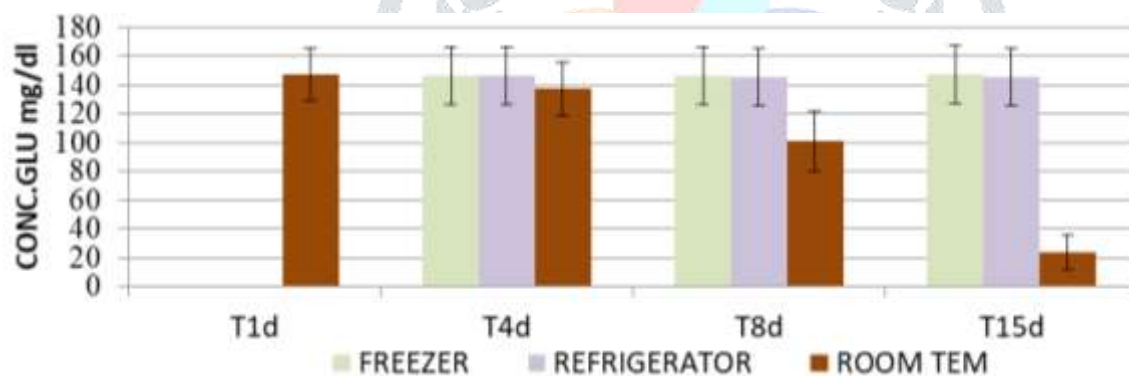

Fig 1: Mean change in glucose concentration when kept at different storage conditions
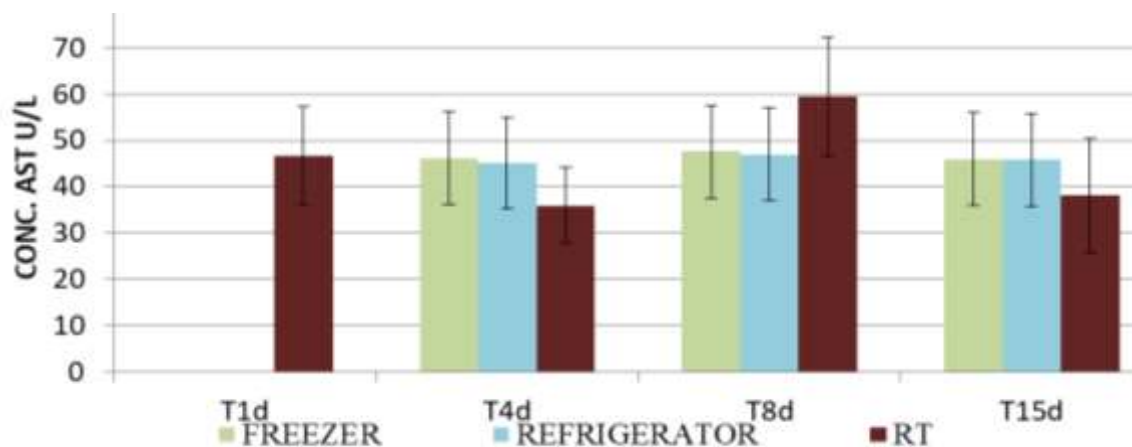

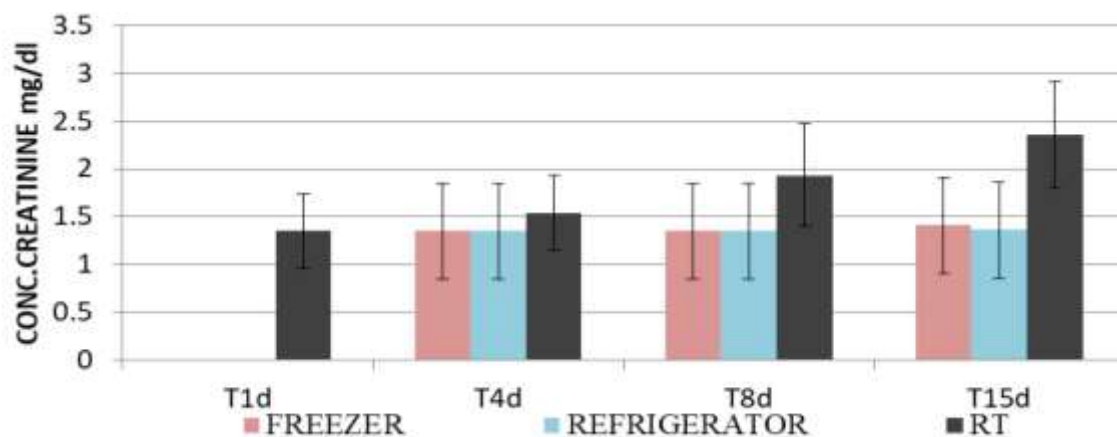Fig 2: Mean change in AST activity in kept at different storage conditions

Fig 3: Mean change in creatinine concentration when kept at different storage conditions

4.DISCUSSION:

The stability is mainly affected by storage time and also by other factors that increase the metabolism of the analytes or cause the initial property to disappear, such as temperature, light, exposure, solvent evaporation or stirring[9]. Hence sample denaturation is clearly temperature and storage duration dependent In general specimens were most susceptible to denaturation at 23 °C[10].

The glycolytic activity of enzymes in plasma and also in presence of formed elements of blood, glucose broken down invitro[11-16]. Lowering temperature to freezing point will decrease enzyme activity as well as growth of microorganisms that might decrease plasma glucose level.

A decline in AST activity noticed for samples at room temperature may be due to increased degradation of enzyme active site with increased temperature, change in the temperature of storage alter the rate of reaction and rate of denaturation of enzymes. Intracellular enzymes are protected from denaturation when bond to their substrates and cofactors. In serum/plasma enzyme substrate and cofactors are dispersed and binding is uncommon, leaving enzyme more susceptible to degradation[17].

The observed findings of this study indicate that all the analytes studied were fairly stable in sample stored at 0 to -8 °C (freezer) and 2 to 8 °C (refrigerator) during the study period, the samples stored at room temperature 23 to 27 °C show significant change in its stability consistent with the previous work of Quartey et al[18] all analyes under study were stable at -60 °C, where creatinine show a significant change when kept at room temperature and refrigerator. Excluding the possibilities of prolonged cell contact previous to centrifugation in this study. Boyanton et al[19] and Marjani et al[20] studied the effect on cell contact, analysis should be done immediately after sample collection the effect of cell contact is more pronounced in plasma. Michael[21] studied the analytes commonly preferred for add on testing and he concluded AST and creatinine were stable upto less than 8 hours and glucose upto 16 hours when stored at 2 to 8 °C. The observed data indicate that, concentrations or activity of certain constituents in human plasma stored under different

conditions will be altered. It is evident that time and temperature have significant effect on the stability of human plasma analytes. Group 1 (0 to -8 ℃) being most stable, Group 2 (2 to 8 ℃) was fairly stable and Group 3 (22 to 27 ℃) the sample stored at room temperature showed least stability among all the analytes under study .

5.CONCLUSION:

The study showed that glucose, AST, and creatinine are most stable in the immediately separated plasma samples following 15 days of storage at freezer (0 to -8 ℃) and fairly stable at refrigerator temperature (2 to 8 ℃). However, there are considerable variations in stability of biochemical analytes in plasma separated samples during storage at room temperature (22 to 27 ℃) and as the time progresses there was concomitant change in analyte concentration, these have to be taken into consideration of prolonged delays in analysis of samples. The term "Instability" or "Deterioration" of biochemical analytes in plasma during storage does not always suggest reduction in concentrations of the analytes as observed in glucose but, in some cases rather unexplained increases as in creatinine. Considering the observation that all the analytes in the samples stored at 0 to -8 ℃ were stable with variations in stability seen in only the room temperature and refrigerator samples with the progress of time, it suggests temperature and time mediated mechanisms as causes of these changes. The observed variations however, are worth investigating, in the light of inconsistencies with previously reported works. Additionally, excluding the effect of cell contact, it is important to investigate and identify the defined biochemical mechanisms underlying the causes of these variations in stability in plasma separated samples during storage conditions. We need to consider specimen sealing, collection tubes, storage temperature changes and specific possible factors in each laboratory. When the result from the sample in different storage conditions and time are analysed, they need to be interpreted accordingly.

REFERENCES

1. Laessig RH, Indriksons AA, Hassemer DJ, Paskey TA, Schwartz TH. Changes in serum chemical values as a result of prolonged contact with the clot. Am J Clin Pathol 1976;66:598-604

2. Boyanton BL Jr., Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242-7.

3. Heins M, Heil W, Withold W. Storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes. Eur J Clin Chem Clin Biochem 1995;33:231-8.

4. Madira WM, Wilcox AH, Barron JL. Storage of plasma in primary plasma separator tubes. Ann Clin Biochem 1993;30(Pt 2):213-4.

5. Felding P, Petersen PH, Hørder M. The stability of blood, plasma and serum constituents during simulated transport. Scand J Clin Lab Invest 1981;41:35-40.

6. Berg B, Estborn B, Tryding N. Stability of serum and blood constituents during mail transport. Scand J Clin Lab Invest 1981;41:425-30.

7. Männistö T, Surcel HM, Bloigu A, Ruokonen A, Hartikainen AL, Järvelin MR, et al. The effect of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid autoantibodies: Implications for analyzing samples stored in serum banks.

8. Kale VP, Patel SG, Gunjal PS, Wakchaure SU, Sundar RS, Ranvir RK, et al. Effect of repeated freezing and thawing on 18 clinical chemistry analytes in rat serum. J Am Assoc Lab Anim Sci 2012;51:475-8.

9. Article in Clinical Chemistry and Laboratory Medicine, published October 2018AuthorsRubén Gómez Rioja, Débora Martínez Espartosa, Marta Segovia, Mercedes Ibarz, María Antonia Llopis, Josep Miquel Bauça, Itziar Marzana, Nuria Barba, Monserrat Ventura, Isabel García del Pino, Juan José Puente, Andrea Caballero, Carolina Gómez, Ana García Álvarez, María Jesús Alsina, Virtudes Álvarez

10. Evaluation of the short-term stability of specimens for clinical laboratory testing.Ikeda K, et al. Biopreserv Biobank. 2015.

11. Hartman, J. D., et al., Comparison of the glycolytic activity of bloods, exudate leucocytes.J. Applied Phy.siol. 12, 477 (1958).

12. Lugalloca, I. 5., et al., Metabolic activity of blood platelets. I)oklady Akad.Nau-. SSSR 118,537 (1958).

13. Mishihara, V., Stored blood glyeolysis. Okapama Igakkai Zasshi 71, 1763 (1959).

14. Beot, C. H., and Taylor, N. B., Physiological Basis of Medical lractice (ed. 6). Williams & \ViIkiIIs, 1955, p. 658.

15. Gradvohl, R. B. H. "Diagnosis" in Bray and Braver, Clinical Laboratory Methods (ed. 5).C. V. Mosby, St. Louis, Mo., 1956, p. 192.

16. Kleiner, I. S., Hurnaa Biochemistry (ed. 2). C. V. Mosby, St. Louis, Mo., .1948, p. 159.

17. Tubular Reabsorption and Secretion Joseph Feher, in Quantitative Human Physiology

18. Stability of Selected Biochemical Analytes in Plasma Samples Stored under Different Time and Temperature Conditions Perez Quartey*, Quartey Perez, Orraca-Tetteh James and Seglah Richard Yawo Department of Medical Laboratory Technology, Garden City University College, Kumasi, Ashanti, Ghana Corresponding author: Quartey P, Garden City University College Kumasi, Ashanti Ghana; E-mail: quartey.perez@gcuc.edu.gh

19. Stability Studies of Twenty-Four Analytes in Human Plasma and Serum Bobby L. Boyanton, Jr., and Kenneth E. Blick , *Clinical Chemistry* 2242—2247 (2002)

20. Marjani. Effect Of Storage Time And Temperature On Some Serum Analytes. The Internet Journal of Laboratory Medicine. 2006 Volume 2 Number 2.

21. Time-Dependent Stability of 22 Analytes in Lithium-Plasma Specimens Stored At Refrigerator Temperature For Up To 4 Days Michael J. Van Vrancken, MD, MPH,* Donna Briscoe, BS, MT(ASCP), Katherine M. Anderson, MA, MT(ASCP), Frank H. Wians Jr, PhD, MT(ASCP)