Section: Biochemistry

Original Research Article

A CROSS-SECTIONAL OBSERVATIONAL STUDY ON THE IMPACT OF CHANGES IN SAMPLE STORAGE CONDITIONS AND TEMPERATURE ON THE ANALYSIS OF CLINICAL BIOCHEMICAL PARAMETERS

Manoj.D[1], Sobia.B.S[2], Sangeetha.T[3], Dheebalakshmi.N[4]

[1]Post Graduate, Department of Biochemistry, Government Coimbatore Medical College, Coimbatore, Tamilnadu, India.
[2]Assistant Professor, Department of Biochemistry, Government Coimbatore Medical College, Coimbatore, Tamilnadu, India.
[3]Assistant Professor, Department of Biochemistry, Government Coimbatore Medical College, Coimbatore, Tamilnadu, India.
[4]Professor and Head, Department of Biochemistry, Government Coimbatore Medical College, Coimbatore, Tamilnadu, India.

Received : 05/10/2025
Received in revised form : 22/11/2025
Accepted : 10/12/2025

Keywords:
Blood Specimen Handling, Specimen Storage, Temperature, Clinical Chemistry Tests, Biochemical Parameters, Preanalytical Phase.

Corresponding Author:
Dr. Dheebalakshmi. N,
Email: biodeepa2007@gmail.com

DOI: 10.47009/jamp.2025.7.6.170

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (6); 917-921

ABSTRACT

Background: Aim: This study aimed to evaluate the impact of storage time and temperature on the test results of common biochemical analytes in serum. **Materials and Methods:** Blood samples (5 mL) were collected from 80 healthy volunteers using clot activator tubes without anticoagulants. After allowing the blood to clot for 30 minutes at room temperature, serum was separated and divided into four aliquots. The first aliquot, analyzed within two hours, served as the baseline value. The remaining three aliquots were stored under different conditions: room temperature 20-25°C for four hours, 2–8°C for four hours, and 2–8°C for 24 hours. **Result:** When baseline serum values analyzed within two hours were compared with samples stored under varying durations and temperatures, the common biochemical analytes such as glucose, urea, creatinine, bilirubin, protein, albumin, cholesterol, triglycerides, HDL, SGPT, SGOT and ALP showed statistically significant changes. The values of blood urea, serum SGOT and serum bilirubin remained nearly stable even with varying storage conditions. **Conclusion:** Based on our findings, storage of serum analytes like glucose, urea, creatinine, bilirubin, protein, albumin, cholesterol, triglycerides, HDL, SGPT, SGOT and ALP is not recommended. Therefore, it is recommended to analyze these samples within two hours of sample collection. **Clinical Significance:** In a patient-centered healthcare approach, clinical laboratories play a critical role. Despite advancements, errors due to insufficient awareness of proper sample collection and storage procedures persist. Improper sample handling can compromise test accuracy, undermine confidence in healthcare services, and harm an institution's reputation. Effective interdepartmental collaboration is essential for identifying and managing preanalytical errors, enabling accurate medical decisions and better patient care.

INTRODUCTION

Laboratory tests are essential for diagnosing and monitoring the treatment of various diseases. The storage duration of blood samples significantly affects their biochemical and physical properties, potentially altering analyte values. These changes, referred to as storage lesions, primarily result from hemolysis. Hemolysis can compromise sample integrity through erythrocyte rupture, the release of intracellular contents into the serum, hemodilution, or alterations in hemoglobin concentration, all of which can directly impact analyte levels. Serum is the preferred sample type for routine assays in clinical laboratories.

To ensure accurate detection of pathological changes, minimizing storage lesions to levels that do not interfere with clinical interpretation is critical. Standard guidelines recommend separating plasma or serum from cells within 20–30 minutes or as soon as clotting is complete to prevent analyte concentration changes caused by clot-cell interactions. Prolonged contact between plasma or serum and cellular

components can lead to inaccurate results due to continued cellular metabolism.

To address this issue, plasma and serum should be separated from cells promptly. While several studies have investigated time-dependent changes in specific analytes, their conclusions often vary. This study focused on evaluating the impact of storage time and temperature on various laboratory analytes, including glucose, total protein, albumin, total and direct bilirubin, AST, ALT, ALP, urea, creatinine, total cholesterol, triglycerides, and HDL. The objective was to assess quantitative changes, determine the optimal duration and temperature for serum storage, and evaluate the clinical significance of these alterations.

MATERIALS AND METHODS

Aim: The study aimed to evaluate the impact of storage duration and temperature on the test results of eight commonly measured biochemical analytes in serum.

Objective: To evaluate the stability of biochemical analytes in serum when stored for different time duration and temperature conditions

Study Design: A cross-sectional observational study comprising of samples collected from volunteers in master health checkup OP Department

Study Period: 2 months

Study Population: Volunteers attending master health checkup OPD, Govt coimbatore medical College Hospital were taken up for the study.

Sample Size: Eighty

Inclusion Criteria: The study included volunteers aged between 20 and 50 years.

Exclusion Criteria: Hemolysed, chyluric and Icteric samples were excluded from the study.

Methodology:

Institutional Ethical Committee approval

↓

After getting informed and written consent

↓

Five mL of venous blood was collected from 80 volunteers visiting master health checkup OPD

↓

Serum was separated as early as possible within two hours from sample collection and made into four aliquots

- I aliquot: The separated serum is analyzed within two hours at room temperature (20–25˚ C) and they act as a baseline value.
- II aliquot: Serum is kept at room temperature (20–25°C) and analyzed after four hours.
- III aliquot: Serum is stored in a refrigerator at 2–8°C and analyzed after four hours.
- IV aliquot: Serum is stored in a refrigerator at 2–8°C analyzed after 24 hours.

↓

Samples will be analysed for Glucose by GOD-POD method, Blood urea by Urease–GLDH Method, serum creatinine by Modified Jaffe'smethod, serum ALP by AMP method, AST by IFCC method, ALT by IFCC method, serum bilirubin by DIAZO Method, serum protein by Biuret method, serum Albumin by BCG Method, serum cholesterol by CHOD-PAP method, triglyceridesby GPO method, HDL by direct enzymatic method using fully automated analyser ERBA XL-640.

↓

Stastical analysis will be done using SPSS software version-30

Potential risk and benefit: There is no increased risk in the study except for routine risk of blood collection.

Outcome measures: Identifying analytes that undergo significant changes under improper storage conditions and those that remain stable despite varying storage conditions helps enhance the precision and accuracy of laboratory diagnostic methods, particularly concerning sample storage duration and temperature.

Stastical Analysis: A p value was derived by applying student T-test using SPSS version 30.

$p < 0.05$ is considered as statistically significant

RESULTS

The comparison of serum analytes measured within two hours at room temperature (20–25°C) with those stored for four hours under the same conditions revealed significant changes, for all the parameters, as shown in Table 1.

Table 1: Changes in the mean value of serum analytes when stored for four hours at room temperature

S.no	Analytes	Sample size	Wthin 2 hours Mean ± SD	4 hours at Room temperature 20-25˚CMean ± SD	p value	Correations
1	Glucose mg/dL	80	122.35±56.83	101.43±65.12	.000	.983
2	Urea mg/dL	80	22.57±6.81	24.73±7.55	.000	.772
3	Creatinine mg/dL	80	0.86±0.18	0.99±0.15	.000	.622
4	Cholesterol mg/dL	80	180.75±33.16	204±48.77	.000	.650
5	Triglycerides mg/dL	80	149.65±87.46	166.63±139.48	.000	.911
6	HDL mg/dL	80	54.73±10.80	47.77±9.37	.000	.639
7	Total bilirubin mg/dL	80	0.66±0.27	0.68±0.30	.000	.986

ISSN (O): 2687-5365; ISSN (P): 2753-6556

8	Direct bilirubin mg/dL	80	0.34±0.11	0.35±0.13	.000	.860
9	SGPT IU/L	80	30.32±16.01	26.90±13.62	.000	.953
10	SGOT IU/L	80	28±19.36	27.88±20.89	.000	.930
11	ALP IU/L	80	89.05±28.51	81.45±24.57	.000	.555
12	Globulin g/dL	80	2.75±0.39	3.40±0.71	.000	.428
13	T Albumin g/dL	80	4.08±0.21	4.49±0.25	.000	.529
14	T Protein g/dL	80	6.84±0.39	7.89±0.79	.001	.363

Table 2: Changes in the mean value of serum analytes when stored for four 4 hours at 2-8˚C

S.no	Analytes	Sample size	Wthin 2 hours Mean ± SD	4 hours at 2-8˚C Mean ± SD	p value	Correations
1	Glucose mg/dL	80	122.35±56.83	141.95±65.06	.000	.993
2	Urea mg/dL	80	22.57±6.81	23.70±7.29	.000	.764
3	Creatinine mg/dL	80	0.86±0.18	1.03±0.14	.000	.620
4	Cholesterol mg/dL	80	180.75±33.16	196.82±40.22	.000	.710
5	Triglycerides mg/dL	80	149.65±87.46	132.95±79.29	.000	.987
6	HDL mg/dL	80	54.73±10.80	44.65±9.18	.000	.912
7	Total bilirubin mg/dL	80	0.66±0.27	0.70±0.28	.000	.987
8	Direct bilirubin mg/dL	80	0.34±0.11	0.32±0.11	.000	.899
9	SGPT IU/L	80	30.32±16.01	26±14.46	.000	.980
10	SGOT IU/L	80	28±19.36	28.52±21.03	.000	.981
11	ALP IU/L	80	89.05±28.51	93.20±25.48	.000	.874
12	Globulin g/dL	80	2.75±0.39	2.30±0.51	.000	.816
13	T Albumin g/dL	80	4.08±0.21	4.40±0.20	.000	.933
14	T Protein g/dL	80	6.84±0.39	6.66±0.46	.000	.846

Table 3: Changes in the mean value of serum analytes when stored for 24 hours at 2-8˚C

S.no	Analytes	Sample size	Wthin 2 hours Mean ± SD	24 hours at 2-8˚C Mean ± SD	p value	Correations
1	Glucose mg/dL	80	122.35±56.83	142.53±63.01	.000	.994
2	Urea mg/dL	80	22.57±6.81	25.25±7.62	.000	.771
3	Creatinine mg/dL	80	0.86±0.18	1.14±0.18	.000	.493
4	Cholesterol mg/dL	80	180.75±33.16	219.95±44.68	.000	.772
5	Triglycerides mg/dL	80	149.65±87.46	190.88±174.99	.000	.939
6	HDL mg/dL	80	54.73±10.80	50.6±10.73	.000	.940
7	Total bilirubin mg/dL	80	0.66±0.27	0.69±0.29	.000	.984
8	Direct bilirubin mg/dL	80	0.34±0.11	0.3±0.11	.000	.886
9	SGPT IU/L	80	30.32±16.01	31.52±16.99	.000	.950
10	SGOT IU/L	80	28±19.36	28.02±22.36	.000	.974
11	ALP IU/L	80	89.05±28.51	73.47±27.64	.000	.846
12	Globulin g/dL	80	2.75±0.39	3.31±0.49	.000	.706
13	Albumin g/dL	80	4.08±0.21	4.65±0.32	.000	.767
14	T Protein g/Dl	80	6.84±0.39	8.02±0.53	.000	.739

In Table 2, the means of analytes tested within two hours were compared with those analyzed after four hours of storage at 2–8°C. Significant differences were noted for all the parameters.

Table 3 presents the comparison of analytes measured within two hours and those stored at 2–8°C for 24 hours. Significant changes ($p < 0.001$) were noted for all the parameters.

DISCUSSION

Minor variations in specimen processing or handling can significantly impact the reliability and reproducibility of analytical results. Serum, the liquid component of blood devoid of cells and clotting factors, typically requires 30–60 minutes to clot at room temperature. Allowing less than 30 minutes for clotting may result in retained cells and contamination, whereas clotting beyond 60 minutes can lead to cell lysis and the release of cellular components.[1]

The Clinical and Laboratory Standards Institute (CLSI) recommends that serum be separated within two hours for most common biochemical analytes to ensure optimal reliability (CLSI, 2023).[2] The storage duration and temperature of serum after separation are also critical, as these factors can influence test outcomes. Laboratories may sometimes adopt improper practices due to heavy sample loads. This study focuses on evaluating the stability of common analytes under various storage conditions.

Selvakumar et al. explored the effects of delayed analysis, ambient temperature, and hemolysis on analytes such as glucose, urea, creatinine, electrolytes, and alkaline phosphatase. Their findings revealed significant changes in serum glucose and alkaline phosphatase levels after four hours and 24 hours of storage at room temperature.[3]

Similarly, Cuhadar et al. investigated the stability of analytes stored in serum gel tubes and plain tubes under different conditions of time and temperature. Their study examined samples stored at 4°C and 24°C for intervals ranging from 6 hours to one week. Results indicated that glucose, total bilirubin, urea

ISSN (O): 2687-5365; ISSN (P): 2753-6556

nitrogen, and uric acid exhibited greater stability when stored at 4°C in gel tubes.[4]

In our study, delays in analysis revealed changes in serum glucose levels, likely due to glycolysis, where glucose is consumed for cellular metabolism. The rate of glucose depletion depends on both temperature and time. Higher temperatures accelerate metabolic activity, leading to faster glucose depletion, while lower temperatures slow this process.[5] Notably, no preservatives were used in the glucose samples analyzed in this study. Sodium fluoride is commonly recommended as a preservative in blood samples for glucose estimation to inhibit glycolysis.[6]

A significant change in urea levels was observed in Table 2, where the mean urea concentration stored for four hours at 2–8°C (23.70 ± 7.29 mg/dL) was compared with urea analyzed within two hours at room temperature (22.57 ± 6.81 mg/dL). This finding contrasts with earlier studies reporting urea stability for up to 15 days when stored at 0–4°C.[7] The increase in urea concentration was statistically significant, it may hold clinical significance, possibly due to the ongoing plasma protein metabolism, by endogenous proteolytic enzymes and thereby increasing blood urea levels correlating readily with storage temperature and storage duration. Additionally, as shown in Table 1 and 3, statistically significant changes in urea levels were observed under other conditions analyzed in this study, with increase in urea concentration on sample storage, probably due to the above mentioned reason.

We observed that serum creatinine levels increased when samples were stored irrespective of temperature and duration, where the instability may be due to positive interference of Jaffe's method from substances like pyruvates, or due to temperature dependant conversion of creatine. This aligns with findings from Shepherd et al., who reported instability in serum creatinine values within 24 hours when measured using Jaffe's method, likely due to interference from substances like ketones and pyruvates.[8]

In contrast with studies by Kachhawa et al. and Comstock et al., serum cholesterol and triglyceride levels were found to increase with storage temperature and storage duration, significantly.[9,10] The reason behind the above changes are increased cholesterol esterases activity and increased free cholesterol levels over increasing storage time and temperature. The reason for reduced Triglycerides level in table 2 scenario is due to the increased lipase activity and degradation of the same with time.

The HDL levels were significantly reduced in all three table scenarios, is due to increased proteolytic apolipoprotein [primarily Apo-1] degradation or may be due to aggregation of HDL at altered pH levels.

In our study, total bilirubin showed significantly increased values when stored at room temperature for four hours and at 2–8°C for 24 hours. Albumin in the serum samples undergo denaturation with storage time and temperature releasing free bilirubin and also bilirubin auto oxidation products interfering with the assay procedure can contribute to the raised total bilirubin levels.

The tables 2 and 3 showed, fall in direct bilirubin levels with storage time and temperature, probably due to increased beta glucuronidase activity, and spontaneous oxidation of direct bilirubin.

In contrast, other studies reported total bilirubin stability for 24 hours.[11] Studies have demonstrated better stability for total bilirubin in gel tubes stored at 4°C. In Lawson et al.'s research total protein was stable in lyophilized samples,[12] but our study found statistical instability when total protein was stored at room temperature. Samples stored at 2–8°C showed better stability. Additionally, changes in serum albumin and globulin were observed across all storage conditions, emphasizing the temperature-dependent nature of protein stability in serum samples, probably due to sample evaporation and conformational changes in proteins. To minimize protein degradation, it is essential to avoid delays in sample processing.[13,14]

In our study the fall in SGPT activity as shown in tables 1 and 2, is due to gradual denaturation of the enzyme with storage time and temperature. In our study the variations in SGOT activity is very minimal inspite of being statistically significant as shown in tables 1, 2, and 3, is might be due to low sample size.

In our study the fall in ALP activity as shown in tables 1 and 3, is due to gradual denaturation of the enzyme with storage time and temperature.

This study provided insights into the optimal storage durations for common serum analytes, helping to establish guidelines for their preservation.

Table 4: Changes in the mean values of serum analytes from within two hours were compared with samples stored under all three durations and temperatures

S.no	*Analytes*	*Sample size*	*Wthin 2 hours Mean ± SD*	*4 hours at 20-25˚C Mean ± SD*	*4 hours at 2-8˚C Mean ± SD*	*24 hours at 2-8˚C Mean ± SD*
1	*Glucose mg/dL*	80	122.35±56.83	101.43±65.12	141.95±65.06	142.53±63.01
2	*Urea mg/dL*	80	22.57±6.81	24.73±7.55	23.70±7.29	25.25±7.62
3	*Creatinine mg/dL*	80	0.86±0.18	0.99±0.15	1.03±0.14	1.14±0.18
4	*Cholesterol mg/dL*	80	180.75±33.16	204±48.77	196.82±40.22	219.95±44.68
5	*Triglycerides mg/dL*	80	149.65±87.46	166.63±139.48	132.95±79.29	190.88±174.99
6	*HDL mg/dL*	80	54.73±10.80	47.77±9.37	44.65±9.18	50.6±10.73
7	*Total bilirubin mg/dL*	80	0.66±0.27	0.68±0.30	0.70±0.28	0.69±0.29
8	*Direct bilirubin mg/dL*	80	0.34±0.11	0.35±0.13	0.32±0.11	0.3±0.11
9	*SGPT IU/L*	80	30.32±16.01	26.90±13.62	26±14.46	31.52±16.99
10	*SGOT IU/L*	80	28±19.36	27.88±20.89	28.52±21.03	28.02±22.36

ISSN (O): 2687-5365; ISSN (P): 2753-6556

11	*ALP IU/L*	80	89.05±28.51	81.45±24.57	93.20±25.48	73.47±27.64
12	*Globulin g/dL*	80	2.75±0.39	3.40±0.71	2.30±0.51	3.31±0.49
13	*T Albumin g/dL*	80	4.08±0.21	4.49±0.25	4.40±0.20	4.65±0.32
14	*T Protein g/dL*	80	6.84±0.39	7.89±0.79	6.66±0.46	8.02±0.53

CONCLUSION

Our study revealed significant changes in common biochemical analytes such as glucose, urea, creatinine, bilirubin, protein, albumin, cholesterol, triglycerides, HDL, SGPT, and ALP when stored under varying durations and temperatures. Therefore, it is recommended to analyze these samples within two hours of collection. Even though blood urea, serum SGOT and serum bilirubin showed statistically significant difference, the values remained nearly stable even with storage. This study highlighted sensitive analytes that are prone to variation under improper storage and identified those that could maintain stability despite variable conditions. These findings contribute to improving the precision and accuracy of diagnostic practices.

Acknowledgment: We, the authors acknowledge the support of the Department of Biochemistry, Government Coimbatore Medical College, Coimbatore, Tamilnadu, India -641018 for providing the necessary facilities and infrastructure to conduct this study.

We also thank the laboratory technical staff of the Department of Biochemistry, Government Coimbatore Medical College, Coimbatore for their assistance in sample processing and analysis.

We also acknowledge the Institutional Ethical Committee of Coimbatore Medical College, Coimbatore for granting ethical approval for this study.

Conflicts of Interest Statement: Authors declare that this is a self-funded study and we have no financial interests or personal conflicts that may affect the study in this article.

Authors declare that we have no financial interests and/or personal relationships that could be seen as a potential conflict of interest.

REFERENCES

1. Timms JF, Arslan-Low E, Gentry-Maharaj A, et al. Clin Chem. PubMed. 2007;53(4):645-656.
2. CLSI document H 18-A3. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline. 3rd edn. 2004.
3. Selvakumar C, Madhubala V. Effect of sample storage and time delay (delayed processing) on analysis of common clinical biochemical parameters. International Journal of Clinical Biochemistry and Research, July-September 2017;4(3):295-298.
4. Cuhadar S, Atay A, Koseoglu M, et al. Stability studies of common biochemical analytes in serum separator tubes with or without gel barrier subjected to various storage conditions. Biochemia Medica 2012; 22(2):202-214.
5. Bruns DE, William C. Knowler. Stabilization of Glucose in Blood Samples: Why It Matters. Clin Chem 2009 Mar;55(5):850-852.
6. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989; 35:315-317.
7. Pahwa M, Menaka K, Minakshi, et al. Effect of storage time and temperature on serum clinical biochemistry analytes. BCAIJ, 2015;9(4):150-156.
8. Shepherd J, Warner MH, Kilpatrick ES. Stability of creatinine with delayed separation of whole blood and implications for eGFR. Ann Clin Biochem2007; 44:384-387.
9. Kachhawa K, Kachhawa P, Varma M, et al. Study of the Stability of Various Biochemical Analytes in Samples Stored at Different Predefined Storage Conditions at an Accredited Laboratory of India. J Lab Physicians 2017 Jan-Mar; 9(1):11-15.
10. Comstock GW, Burke AE, Norkus EP, et al. Effects of repeated freeze-thaw cycles on concentrations of cholesterol, micronutrients, and hormones in human plasma and serum. ClinChem 2001; 47:139-142.
11. Sofronescu AG, Loebs T, Zhu Y. Effects of temperature and light on the stability of bilirubin in plasma samples. Clin Chim Acta. 2012 Feb;18;413(3-4):463-466.
12. Lawson NS, Haven GT, Moore TD. Long-term stability of enzymes, total protein, and inorganic analytes in lyophilized quality control serum. Am J Clin Pathol. 1977 Jul; 68(1 Suppl):117-129.
13. Mitchell BL, Yasui Y, Li CI, et al. Cancer Inf. PubMed. 2005;1(1):98-104.
14. Rai AJ, Gelfand CA, Haywood B, et al. Proteomics. PubMed. 2005;5(13):3262-3277.

ISSN (O): 2687-5365; ISSN (P): 2753-6556