



Analysis of Preanalytical Variable – Stability of Common Biochemical Analytes under Different Storage Conditions and Temperatures

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Abstract

Aim: To evaluate the stability of 11 common biochemical analytes in serum samples under different storage conditions and temperatures. **Materials and Methods:** 5ml of venous blood were collected from each volunteer into sterile clot activator tubes. The samples were allowed to clot for 30 minutes at room temperature and centrifuged at 2500 rpm for 10 minutes. Serum was separated as early as possible within two hours from sample collection and made into four aliquots and analysed using XL 640 biochemical autoanalyzer. The first aliquot analysed within two hours act as a baseline value. The remaining three aliquots were stored as follows: Room temperature (20-25 ° C) for four hours, refrigerated at 2-8° C for four hours and refrigerated at 2-8° C for 24 hours, respectively. **Results:** Baseline serum values of biochemical analytes analyzed within two hours were compared with those stored under different conditions. Samples stored at room temperature for four hours showed a significant change in the values of glucose, urea, creatinine, total bilirubin, albumin, total protein, AST, ALT and ALP. Refrigerated at 2-8° C for four hours caused significant changes in glucose, urea and albumin. Storage at 2-8° C for 24 hours led to significant change among the values of glucose, total bilirubin, and albumin. Other analytes were found to be stable across different conditions. **Conclusion:** The study revealed that certain serum analytes like glucose, urea, bilirubin, and albumin are particularly sensitive to storage time and temperature, emphasizing the need for prompt analysis— ideally within 2 hours of collection. In contrast, serum creatinine, total protein, AST, ALT and ALP were stable on refrigeration. Total cholesterol and triglycerides remained stable under all tested conditions. These findings help enhance the accuracy of diagnostic practices by identifying which analytes are more affected by improper storage. **Clinical Significance:** In the framework of patient-centered healthcare delivery, clinical laboratories play a pivotal role in ensuring accurate diagnosis and effective treatment. While technological advancements have enhanced laboratory processes, preanalytical errors— particularly those arising from improper sample collection, handling, and storage—remain a persistent challenge. Such errors can compromise test results, leading to reduced confidence in healthcare services and potentially harming the institution's credibility. Timely identification and correction of these errors are essential to safeguard the accuracy and reliability of laboratory data. Fostering effective interdepartmental collaboration is crucial to minimize preanalytical issues, thereby enhancing clinical decision-making and ultimately improving patient outcomes.

Keywords: Accuracy, Aliquot, Analytes, Preanalytical Variables, Precision

1. Introduction

Physicians and clinicians use laboratory test to aid in the diagnosis, monitoring, prognosis and follow up of diseases as we live in an era of evidence-based medicine. Hence precision and accuracy of a test result

are important. Several steps in the procedure include patient preparation, sample collection, processing of samples, analysis, reporting, and interpretation of test results. The sources of errors that affect the accuracy of test results are classified into preanalytical, analytical and postanalytical^{1,2}.

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The total testing process includes preanalytical, analytical, and postanalytical phases. The preanalytical phase is the primary source of laboratory mistakes in clinical laboratories. Many preanalytical variables like specimen storage time, specimen storing temperature can be monitored and controlled thereby reducing the magnitude of the errors and improving the accuracy of the test result

The International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Laboratory Errors and Patient Safety (IFCC WG-LEPS) created a list of quality indicators for reducing errors in the laboratory as well as 16 quality indicators related to important preanalytical steps required for the reliability of laboratory results, such as proper blood collection, the correct specimen type, and timely processing etc.

The information available for sample stability is incomplete and confusing, especially under different storage conditions and temperatures. Many preanalytical variables like specimen storage time, specimen storing temperature can be monitored and controlled thereby reducing the magnitude of the errors and improving the accuracy of the test result. The main objective of this study is to evaluate different storage conditions and temperatures influence the concentrations of some selected parameters in a clinically significant way.

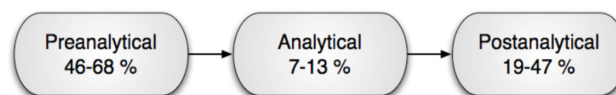
2. Aims and Objectives

- To evaluate the stability of 11 common biochemical analytes in serum samples under different storage conditions and temperatures. significant way.
- To assess the effect of different storage temperatures (Room Temperature, refrigeration) on the stability of 11 common biochemical analytes.
- To determine the Time dependent variability in 11 common biochemical analytes during storage under controlled condition.
- To prove any significant variation in the stability of 11 common biochemical analytes in serum samples to reduce preanalytical error.

3. Review of Literature

Pre analytical variability is defined as errors which occur when non analytical factors change the concentration

Errors within the total testing process



Pre-analytical Errors

Pre-Analytical Errors	Major Sources
Test request	Erroneous request, inappropriate request
Patient identification	Wrong or missing identification
Patient preparation and condition	Biological variables, diet, fasting vs. non-fasting
Time of specimen collection	Diurnal, fasting, peak and trough
Specimen collection and identification	Mislabeled or unlabeled, hemolysis, incorrect order of draw, wrong tube type/sample/ anticoagulant, IV line (contamination from infusion), tourniquet time, posture, fist clenching, traumatic draw, insufficient or vigorous tube mixing, quantity not sufficient (QNS)
Sample transportation	Length and environmental conditions, excessive transportation time, temperature, pneumatic tube system
Sample storage	Improper storage, length of time, temperature, evaporation, freezing and thawing



Figure 1. Pre-analytical Errors.

of analytes, so that the results do not reflect correctly the condition of the patient. Preanalytical variables consists of Precollection variables, Sample collection variables and Postcollection variables.

The human body is composed of many different compound and elements; the concentration or activity of these analytes in body fluids may reflect an individual's health or pathophysiological state. Many factors other than disease may affect the concentration or activity of these analytes

Thus, laboratories are responsible for taking steps to minimize sources of error by developing standard

procedures for the control of pre analytical errors particularly postcollection part.

Preanalytical variability accounts for the majority of laboratory errors, with estimates suggesting it contributes to nearly 60-70 % of total diagnostic inaccuracies (Lippi *et al.*, 2011). Among these, improper sample storage and delayed processing are significant contributors to altered biochemical analyte values.

The stability of biochemical parameters is influenced by factors such as temperature, duration before analysis, and sample handling. Studies have demonstrated that glucose levels decline over time at room temperature due to glycolysis, even when using fluoride-containing tubes (Chan *et al.*, 1989)⁹. Refrigeration has been shown to slow but not completely prevent this decline (Svensson *et al.*, 2012).

Proteins such as albumin and total protein are relatively stable under refrigerated conditions but can show degradation at higher temperatures or with prolonged storage (Boyanton and Blick, 2002). Similarly, bilirubin is photosensitive and susceptible to oxidative degradation, with significant reductions observed when samples are exposed to light and stored for extended periods (Doumas *et al.*, 1973).

Enzymatic activities, including AST, ALT, and ALP, may remain stable for several days under refrigeration at -20 deg celsius, but extended storage can result in conformational changes leading to loss of enzymatic activity (Guder *et al.*, 2009). In contrast, analytes such as cholesterol and triglycerides have been found to maintain stability for longer durations under varied storage conditions (Oddoze *et al.*, 2012).

These findings highlight the importance of defining specific preanalytical protocols for different biochemical analytes to ensure accuracy in clinical decision-making. Current guidelines, such as those by the Clinical and Laboratory Standards Institute (CLSI), recommend minimizing the time between sample collection and analysis, and implementing appropriate storage conditions tailored to the analyte's stability profile.

4. Materials and Methods

A cross-sectional study was undertaken in the Department of Biochemistry, Government Chengalpattu Medical College, Chengalpattu, Tamil

Nadu, India. Eighty healthy volunteers of age group 18–60 years visiting master health checkup OPD in our hospital were taken up for the study after getting proper informed consent. The study was approved by the ethical committee of our college.

4.1 Inclusion Criteria

- Healthy volunteers of age group 18–60 years visiting Master Health Checkup OPD.
- Healthy volunteers who give informed consent.

4.2 Exclusion Criteria

- Age group <18 years.
- Critically ill patients and antenatal mothers
- Hemolysed, Icteric and Lipemic samples.

5. Methodology

5ml of venous blood were collected from each volunteer into sterile clot activator tubes. The samples were allowed to clot for 30 minutes at room temperature and centrifuged at 2500 rpm for 10 minutes. Serum was separated as early as possible within two hours from sample collection and made into four aliquots and analysed using XL 640 biochemical autoanalyzer.

- I aliquot: The separated serum is analysed 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 analysed after four hours.
- III aliquot: Serum is stored in a refrigerator at 2–8°C and analysed after four hours.
- IV aliquot: Serum is stored in a refrigerator at 2–8°C analysed after 24 hours.

Each aliquot will be analysed for the following parameters as mentioned in the table 1.

Plasma Glucose, Blood Urea, Serum Creatinine, Total Bilirubin, Total Protein, Albumin, Total Cholesterol, Triglycerides (TGL), AST, ALT, ALP.

Serum was analyzed for eleven common biochemical parameters like Glucose, Blood Urea, Serum Creatinine, Total Bilirubin, Total Protein, Albumin, Total Cholesterol, Triglycerides (TGL), AST, ALT, ALP from all the four aliquots. The parameters were assayed using XL system packs from Erba

diagnostics (Trans Asia Biomedicals Ltd, south sikkim, in technical collaboration with ERBA Diagnostics Mannheim GmbH, Mallaustr, Mannheim/ Germany). Quality control values for the analytes that we studied were within ± 2 SD (standard deviation) of their respective target means.

5.1 Glucose Measurement (GOD-POD Method)

The enzyme glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. The enzyme peroxidase catalyzes the coupling of 4 aminoantipyrine with phenolic compound to yield a colored quinoneimine complex and its absorbance was determined at the wavelength of 505 nm.

5.2 Urea Measurement (Urease–GLDH Method)

Urea is hydrolyzed by urease to ammonia and CO_2 . In the presence of (GLDH) glutamate dehydrogenase, ammonia combines with α -ketoglutarate to form L-glutamate and its absorbance was determined at the wavelength of 340 nm.

5.3 Creatinine Measurement (Modified Jaffe's)

Creatinine reacts with alkaline picrate to yield an orange-red colored complex whose absorbance was measured at 510 nm.

5.4 Triglyceride Measurement (GPO Method)

Triglycerides are hydrolyzed by lipoprotein lipase to glycerol and free fatty acid. Glycerol is phosphorylated by ATP with glycerol kinase to produce glycerol-3-phosphate and ADP. Addition of the enzyme glycerol-3-phosphate oxidase results in the formation of dihydroxyacetone phosphate and H_2O_2 . H_2O_2 reacts with N-ethy-1-N-sulfohydroxypropyl-m-toluidine and 4-aminoantipyrine catalyzed by peroxidase to form a quinoneimine colored compound and its absorbance was determined at the wavelength of 546 nm.

5.5 Albumin Measurement (BCG Method)

Serum albumin reacts with Bromocresol Green (BCG) to form a blue green coloured compound and its absorbance was measured at the wavelength of 630 nm.

5.6 Total Protein Measurement (Biuret method)

The coordination complex formed between the cupric ions and the nitrogen of the peptide bond gives a violet colour product whose absorbance was measured at 540 nm.

5.7 Total Cholesterol Measurement (Cholesterol Oxidase Method)

Cholesterol ester is converted to cholesterol and fatty acid by the enzyme cholesterol esterase. Addition of the enzyme cholesterol oxidase results in the formation of 4-cholesten-3-one and H_2O_2 . A colored compound is obtained on addition the enzyme peroxidase, phenol, and 4-aminoantipyrine whose absorbance was determined at the wavelength of 505 nm.

5.8 Total Bilirubin Measurement (Diazo Method)

Bilirubin is coupled with diazotized sulfanilic acid in the presence of ethylene glycol and dimethyl sulfoxide to produce a coloured dye whose absorbance was measured at 546 nm.

5.9 Aspartate Amino Transferase (AST/SGOT) – Modified IFCC Method

L-Aspartate reacts with α -ketoglutarate and to form oxaloacetate and L-Glutamate in the presence of Aspartate aminotransferase. Malate dehydrogenase reduces Oxaloacetate to malate, using NADH, converting it to NAD^+ . The decrease in NADH which absorbs light at 340 nm is measured. Sample pyruvate is converted to lactate by Lactate dehydrogenase.

5.10 Alanine Amino Transferase (ALT/SGPT) – Modified IFCC Method

L-Alanine reacts with α -ketoglutarate and to form pyruvate and L-Glutamate in the presence of Alanine aminotransferase. Lactate dehydrogenase reduces pyruvate to L-Lactate, using NADH, converting it to NAD^+ . The decrease in NADH which absorbs light at 340 nm is measured.

5.11 Alkaline Phosphatase (ALP) – AMP Method

4-NPP is reacts with AMP and produce 4-nitrophenol and phosphate by alkaline phosphatase enzyme. At the

alkaline Ph, 4 nitrophenol has an intense yellow colour. The reagent also contains a metal ion buffer system that optimal concentrations of Mg^{2+} and Zn^{2+} are maintained. The reaction is monitored by measuring the rate of increase in absorbance at 405nm which is proportional to the activity of ALP in the serum.

6. Statistical Analysis

- Data will be entered in Microsoft excel and analyzed using SPSS software.
- The data will be expressed in terms of mean and standard deviation. Data will be assessed using RM ANOVA test.
- 'P' value less than 0.05 will be taken as the significant value.

7. Results

In comparison of the means of the serum analytes analysed within two hours at room temperature (20–25° C) to that of the sample stored for four hours at room temperature showed a significant change as per Table 2 in the values of glucose (p value 0.005), urea (p value < 0.001), creatinine (p = 0.005), total bilirubin (p value < 0.001), albumin (p = < 0.001), total protein (p = 0.001) AST (p = < 0.001), ALT (p = 0.005) and ALP (p = < 0.001). In the analysis of total cholesterol and triglycerides, there was no significant change.

Table 1. Parameters

Biochemical Parameter	Method
Glucose	GOD-POD Method
Urea	Urease- GLDH Method
S. Creatinine	Modified Jaffe's method
Total bilirubin	Diazo method
Total protein	Biuret method
S. Albumin	Bromocresol green (BCG) method
Total cholesterol	Cholesterol-oxidase method
S. triglyceride	GPO Method
AST(SGOT)	Modified IFCC method
ALT(SGPT)	Modified IFCC method
ALP	AMP method

In Table 3, the means of test values of sample analytes that are analyzed within two hours were compared with the means of the analytes analyzed within four hours after storing at 2–8°C. Table 3 showed significant changes in the p values of glucose (p value < 0.001), urea (p value < 0.001) and albumin (p = 0.002). Other analytes showed no significant change.

Comparison of the means of sample analyzed within two hours and the samples stored at 2 to 8° C for 24 hours was shown in Table 4. A significant change of p value < 0.001 was observed in the value of albumin. Significant p value < 0.05 was observed in values of glucose, total bilirubin, and. Other analytes were not significantly affected when stored at 2–8°C as shown in Table 4.

Table 2. Changes in the mean value of serum analytes when stored for four hours at room temperature

S.no	Analytes	Sample size	Within 2 hours Mean \pm SD	4 hours at RT Mean \pm SD	p value
1	Glucose mg/dL	80	120.65 \pm 65.23	101.48 \pm 55.70	0.005
2	Urea mg/dL	80	25.94 \pm 9.74	32.21 \pm 10.17	< 0.001
3	Creatinine mg/dL	80	1.30 \pm 0.61	1.51 \pm 0.51	0.005
4	Cholesterol mg/dL	80	187.61 \pm 43.92	190.12 \pm 43.82	0.887
5	Triglycerides mg/dL	80	160.64 \pm 90.29	159.65 \pm 90.60	0.939
6	Total bilirubin mg/dL	80	0.58 \pm 0.42	0.37 \pm 0.36	< 0.001
7	T Albumin g/dL	80	3.14 \pm 0.21	3.51 \pm 0.22	< 0.001
8	T Protein g/dL	80	7.29 \pm 0.72	6.12 \pm 0.73	< 0.001
9	AST/SGOT	80	34.22 \pm 7.99	38.31 \pm 10.12	< 0.001
10	ALT/SGPT	80	42.24 \pm 9.55	46.45 \pm 11.24	0.005
11	ALP	80	108.12 \pm 25.20	130.00 \pm 32.45	< 0.001

p < 0.05 statistically significant

Table 3. Changes in the mean value of serum analytes when stored at 2–8°C for 4 hours

S.no	Analytes	Sample size	Within 2 hours Mean \pm SD	4 hours at 2–8° C Mean \pm SD	p value
1	Glucose mg/dL	80	121.65 \pm 60.13	106.4 \pm 57.66	< 0.001
2	Urea mg/dL	80	26.93 \pm 10.24	19.37 \pm 9.80	< 0.001
3	Creatinine mg/dL	80	1.15 \pm 0.55	1.13 \pm 0.48	0.082
4	Cholesterol mg/dL	80	191.61 \pm 44.72	192.81 \pm 43.79	0.268
5	Triglycerides mg/dL	80	161.74 \pm 90.09	160.80 \pm 87.78	0.494
6	T bilirubin mg/dL	80	0.55 \pm 0.44	0.55 \pm 0.44	0.987
7	T Albumin g/dL	80	3.58 \pm 0.19	3.63 \pm 0.17	0.002
8	T Protein g/dL	80	7.26 \pm 0.52	7.30 \pm 0.63	0.583
9	AST/SGOT	80	34.22 \pm 7.99	33.98 \pm 7.39	0.843
10	ALT/SGPT	80	42.24 \pm 9.55	41.25 \pm 9.05	0.502
11	ALP	80	108.12 \pm 25.20	110.10 \pm 25.32	0.620

p < 0.05 statistically significant

Table 4. Changes in the mean value of serum analytes when stored at 2–8°C for 24 hours

S. no.	Analytes	Sample size	Within 2 hours Mean \pm SD	24hours at 2–8° C Mean \pm SD	p value
1	Glucose mg/dL	80	121.65 \pm 60.13	107.74 \pm 59.03	<0.050
2	Urea mg/dL	80	26.93 \pm 10.24	26.93 \pm 10.65	1.000
3	Creatinine mg/dL	80	1.15 \pm 0.55	1.13 \pm 0.48	0.310
4	Cholesterol mg/dL	80	191.61 \pm 44.72	191.54 \pm 44.11	0.950
5	Triglycerides mg/dL	80	161.74 \pm 90.09	160.50 \pm 90.19	0.411
6	Total bilirubin mg/dL	80	0.55 \pm 0.44	0.41 \pm 0.44	0.005
7	T Albumin g/dL	80	3.58 \pm 0.19	3.76 \pm 0.17	<0.001
8	T Protein g/dL	80	7.26 \pm 0.52	7.26 \pm 0.79	0.966
9	AST/SGOT	80	34.22 \pm 7.99	32.98 \pm 7.39	0.198
10	ALT/SGPT	80	42.24 \pm 9.55	39.25 \pm 9.05	0.063
11	ALP	80	108.12 \pm 25.20	106.10 \pm 25.32	0.567

p < 0.05 statistically significant

8. Discussion

Minor changes in specimen processing or handling can have dramatic effects in analytical reliability and reproducibility of test results. Serum is the liquid portion of the blood devoid of cellular elements and clotting factors. Blood samples require 30–60 minutes to clot at room temperature. When the serum samples were allowed less than 30 minutes then, retained cells and contamination can occur. When the serum samples were allowed more than 60 minutes, cellular components may be released due to cell lysis³. Ideal time of serum separation from the sample for most of

the common biochemical analytes is within two hours as per the recommendations of Clinical and Laboratory Standards Institute⁴. The time of storage of serum after separation and the temperature at which they are stored affect the test results. Improper methods may be followed in the laboratory due to the increase in sample load. Our study aims at identifying the stability of some common analytes when subjected to different storage conditions and temperature.

In the study conducted by Selvakumar *et al.*, time delay in the analysis, the effect of ambient temperature and the effect of hemolysis are studied for common analytes like glucose, urea, creatinine, electrolytes and Alkaline

phosphatase. Their results showed a significant change in serum glucose and Alkaline phosphatase levels after four hours and 24 hours at room temperature⁵. In the study conducted by Cuhadar *et al.* aims at determining the stability of analytes in serum gel tubes and plain tubes when subjected to varying storage time and temperature. The samples were stored at 4 or 24°C for 6, 12, 18, 24, 30, 36, 48 and 72 hours and 1 week. In their study glucose, total bilirubin, urea nitrogen and uric acid stored at 4°C in gel tubes showed more stability⁶.

In this study, delay in the analysis showed changes in serum glucose which could be attributed to its utilization by glycolysis. Glucose is required for cellular metabolism and the rate at which glucose is depleted is dependent on temperature and time. At higher temperatures, there is a higher metabolic rate and glucose is depleted quickly, whereas at lower temperatures it is depleted more slowly^{7,8}. No preservatives were added in the glucose sample in our study. Sodium fluoride is preferred as a preservative added to blood samples collected for glucose estimation to prevent glycolysis⁹.

Stability of serum creatinine was noticed when the samples were stored at 2–8°C. Instability was noticed in the sample kept at room temperature. As per the study conducted by Shepherd *et al.* instability among serum creatinine values were reported within 24 hours using Jaffe's method for estimation which was attributed to some interference like ketones and pyruvates¹⁰.

A significant change was observed in urea levels as per Table 2 in which the mean urea concentration stored for four hours at 2–8°C (25.37 ± 9.80 mg/dL) was compared to urea analyzed within two hours at room temperature (26.93 ± 10.24 mg/dL). This was in contrast to some study where urea sample was found to be stable for 15 days when stored at 0–4°C¹¹. The decrease in urea concentration may not be considered as clinically significant though there is statistical significance. This could be attributed to the smaller sample size. As per Table 3, we did not observe any statistically significant change.

In accordance with the study conducted by Kachhawa *et al.* and Comstock *et al.*, serum cholesterol and triglyceride concentrations were found to be stable¹². In our study, total bilirubin showed significant variation when stored at room temperature for four hours and 24 hours at 2–8°C¹³. We placed the samples

in artificial light at room temperature and in the refrigerator without any protection. Total bilirubin was found to be stable for 24 hours in other studies^{14,15}. Stability was found to be better in gel tubes stored at 4°C for total bilirubin in the study conducted by Serap.

Total protein in the lyophilized sample was found to be stable in the study conducted by Lawson *et al.*,¹⁶ but in our study, statistical instability was observed when total protein was stored at room temperature, other samples stored at 2–8°C were stable. We also observed changes in serum albumin in all the stored samples. The stability of proteins in serum samples are temperature dependent. Steps should be taken to prevent delay in sample processing to minimize the degradation of proteins.^{17,18} AST and ALT was found to be stable in our study when it was properly refrigerated at 2–8°C. This was supported by study conducted by Mitchell and Gelfand *et al.*¹⁹ This study helped us to identify the useful length of storage time for common serum analytes.

9. Summary and Conclusion

In this study, we observed significant alterations in several common biochemical analytes in serum—particularly glucose, urea, bilirubin, and albumin—when stored under varying durations and temperatures. These findings underscore the importance of timely processing, and we recommend that such analytes be analyzed within 2 hours of sample collection to ensure result accuracy.

Serum creatinine, total protein, AST, ALT, and ALP demonstrated no statistically significant changes when stored under refrigeration, indicating better stability under controlled conditions. Additionally, total cholesterol and triglycerides remained stable even after extended storage at room temperature, suggesting they are less sensitive to pre-analytical delays.

This study highlights the need to identify temperature- and time-sensitive analytes that are prone to degradation or fluctuation during storage. Our results contribute to optimizing pre-analytical handling protocols and selecting appropriate storage strategies. By identifying analytes with greater susceptibility to instability, laboratories can improve the precision, reliability, and clinical utility of diagnostic test results.

10. Study Limitation

- Single centre study
- This study is cross sectional and couldn't establish causality.
- Sample size was small.
- Run to run variation was not studied.
- Analysis was not done in duplicates to ensure the reliability of results.

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