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Analysing the Stability of Various Biochemical Analytes in Samples Stored at Different Predefined Storage Conditions at a Tertiary Care Hospital Laboratory

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ABSTRACT

Objectives:

1) To estimate stability of biochemical analytes in a samples stored at different predefined storage conditions.
2) To evaluate the stability of biochemical analytes in serum when stored for different time duration.

Material & Methods: This hospital-based study included 30 random samples from outpatients being treated at **Government Medical College & Hospital Akola** during the period of 2 months from August 2023 to October 2023.

A total of 15 biochemical analytes in the sera of 30 patients were examined following storage. Subsequent to determining the baseline measurements; the serum of each patient was aliquoted and stored at -20 ^{0}C for 7,15 and 30 days and analysed for the stability on respective days.

The results were compared with the initial analysis measurements obtained from fresh samples. Mean changes compared to baseline (T0) concentrations were evaluated both statistically & clinically.

Results: The results shows that urea, creatinine, total bilirubin, direct bilirubin, sodium, potassium , lipase, cholesterol, triglycerides, uric acid & lactate dehydrogenase levels were stable under all conditions. Serum amylase was the only analyte demonstrating instability following prolonged storage ; amylase levels changed significantly at 15 & 30 days (p value -0.005). However, serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase were also demonstrating instability following prolonged storage but these changes were not statistically or clinically significant.

Conclusion: Stability of different biochemical parameters were assessed on the basis of result. The study was also indicates whether deep freezing (-20^{0}c) can be useful tool for additional analysis time for patients

care and research purposes.

Keywords: biochemical analyte, serum, stability

INTRODUCTION:

In the era of Evidence based medicine (EBM), clinical laboratories play an inevitable role in screening, diagnosing & prognostic monitoring of diseases in human beings.[1] In medical labs, storage of whole blood & other blood products such as serum or plasma is often necessary due to technical challenges or to reserve samples for future reasons such as research . The main challenge in clinical laboratories is analytes stability in serum/plasma if there is a delay in laboratory examination.[2] Samples are usually stored in the laboratory at different storage conditions, 4-8°C (in the refrigerator) and in deep freezers (-20°C,-40°C, -70°C or -80°C) depending on the need of storage time. Thus the temperature at which the samples are stored is one of the critical parts of pre-analytical quality assurance and it plays a great role to maintain analytes stability or measurement precision.[3]

The sources of errors that affect the accuracy of test results are classified into preanalytical, analytical and postanalytical. **Preanalytical** errors are the most common errors in the 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 .[4’5]

It is important to retain the stability of a sample for a particular period of time and temperature limits of storage. [6] This period should neither be longer nor shorter, to avoid significant changes in the concentrations of the estimated biochemical parameters. The study was designed to analyze the serum samples for urea, creatinine, total bilirubin, direct bilirubin, sodium, potassium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, lipase, cholesterol, triglycerides, uric acid & lactate dehydrogenase at different time intervals and at proper storage conditions. The data collected from the study was implemented in estimation of certain biochemical parameters with appropriate time and at proper storage conditions to avoid analytical errors and also in defining acceptable time delay and storage conditions when a shorter time between sample collection and processing is not possible.

However, limited information is available regarding the stability of commonly used clinical biochemical analytes in human serum including the effect of storage temperatures as low as −20°C on blood-separated serum. Therefore, the present study examined the stability of 15 routine chemistry analytes in immediately cell-separated serum following storage at a designated temperature (−20°C) for different periods (0, 7, 15, and 30 days) using the previously described standard guidelines for blood sample handling and separation.[7]

There have been various studies conducted on this basis, with results specific for the particular conditions of the laboratory, in which the study was conducted. There is a need for each and every clinical laboratory to study on the stability of the samples processed in their premise, for a better reporting of results.

MATERIALS AND METHODS:

Study design

This hospital-based **cross- sectional study** included thirty random samples from outpatients being treated at **Government Medical College & Hospital, Akola**; during the period of 2 months from **August 2023 to October 2023** . The samples collected from each patient were for physician-ordered laboratory testing;

no additional blood was taken from the subjects. The study was started after approval of Institutional Ethical Committee, and informed consent was obtained from all the participants.

Selection Criteria:

Inclusion Criteria:

- Normal healthy volunteers of age group 25–60 years (male & females) from the outpatients being treated at a tertiary care hospital.

Exclusion Criteria:

- Subjects with Diabetes and Hypertension.
- Subjects with history of heart ,kidney & Liver disorder.
- Critically ill patients, children, and antenatal mothers.
- Subjects with any other chronic illness which affect the study results.

Sample collection and analysis

After obtaining written/informed consent from subject ,under all aseptic precautions following a fast of 12 hours, 5-10 ml of venous sample were collected from the antecubital vein of the subjects. Blood samples were centrifuged at 3000rotation per minutes for at least 10 minutes to separate serum and plasma. The separated serum were processed immediately or stored at -20^{0}C.

Serum samples were examined for hemolysis and lipemia to prevent possible interference. The serum samples of each subject were pooled into a plain tube and then aliquoted into 1.5 mL Eppendorf tubes; four aliquots per patient samples were kept (three for storage at −20°C) and the remaining serum was used for the baseline measurement (T1d). All samples were kept frozen until experimental analysis. The serum aliquots were stored frozen at −20°C for either 7 (T7d), 15 (T15d), or 30 (T30d) days and then analyzed separately for stability.

The following analytes were examined: [Table 1]

- Metabolites: Na+, K+, urea, creatinine, uric acid, direct bilirubin, and total bilirubin
- Lipids: Total cholesterol and triglycerides
- Enzymes: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), amylase, lipase and lactate dehydrogenase(LDH).

All the biochemical assay were performed on **Fully automated Auto-Analyser XL-640 by Transsasia**, except for serum electrolyte levels, which were done on **Easylyte Electrolyte Analyser.**

Statistical analysis

To determine time-dependent changes in cell-separated serum analytes, the mean value from all thirty subject samples was calculated for each analyte at each time point. The statistical analysis of the results was made by using Statistical software (SPSS version 21.0) ANOVA. **P value <0.05** considered as statistically significant

RESULTS:

The analysis results for **15 biochemical parameters** measured in serum samples under different storage conditions are shown in Table 2. No significant statistical or clinical differences were found among most of the **metabolites** (Na+, K+, urea, creatinine, uric acid, direct bilirubin, and total bilirubin) under the different storage conditions. The changes in **K+** level at T15d were statistically but not clinically

significant. **Serum alkaline phosphatase** also demonstrated statistically significant variation on T30d compared with fresh sera on T1d; however, this variation was not clinically significant. Aside from **serum amylase**, none of the enzymatic parameters exhibited clinically significant reduction inactivity. The effect of storage at lower temperatures on serum amylase was statistically as well as clinically significant. The changes in **serum cholesterol and serum triglyceride** were statistically significant on T15d and T7d, respectively, compared to T1d; however, they were not clinically significant. This may be attributed to systematic errors occurring on the respective days.

DISCUSSION:

Metabolites :

The results of our study indicate that almost all of the examined metabolites were stable even after 30 days of storage at −20°C. Consistent with the findings of Zhang et al., [7] no clinically significant differences were found for sodium levels following different storage durations compared to fresh samples.[2,7]

Table 1: Methods used for measuring biochemical parameter:

Analysis	Methods
Urea, mg/dL	GLDH kinetic method
Creatinine, mg/dL	Modified Jaffe's method
Total bilirubin, mg/dL	Diazo method
Direct bilirubin, mg/dL	Diazo method
Sodium, mmol/L	ISE
Potassium, mmol/L	ISE
ALP, U/L	Kinetic IFCC
Amylase, U/L	Direct substrate method
Lipase, U/L	Direct substrate method
Total cholesterol, mg/dL	CHOD-POD
Triglyceride, mg/dL	GPO-PAP
Alanine transaminase, U/L	Modified IFCC
Aspartate transaminase, U/L	Modified IFCC
Uric acid, mg/dL	Uricase method
LDH, U/L	Modified IFCC

ALP:Alkaline phosphatase, PAP: Peroxidase antiperoxidase, GPO: Glycerol 3 phosphate oxidase, CHOD-POD: Cholesterol oxidase–peroxidase, ISE: Ion selective electrodes, IFCC: International federation of clinical chemistry

Table 2: Statistical analysis of selected analytes; serum samples were stored at −20°C for 7 (T7d), 15 (T15d), or 30 (T30d) days and then analyzed at room temperature

SR.NO	Parameter	DAY1	DAY 7	DAY 15	DAY30	P value
1	UREA	22.2±6.1	20.0±6.0	19.4±5.7	18.8±5.5	0.5
2	CREATININE	1±0.4	0.8±0.3	0.8±0.3	0.8±0.3	0.1
3	TOTAL BIL	0.9±0.3	0.8±0.3	0.8±0.2	0.8±0.3	1
4	DIRECT BIL	0.34±0.15	0.13±0.14	0.41±0.15	0.15±0.13	0.4
5	SODIUM	140±5.40	139.77±5.46	138.47±5.82	139.54±5.65	0.8
6	POTASIUM	3.89±0.38	3.47±0.37	**4.24±0.37**	3.67±0.37	0.8
7	**ALP**	124.0±8.44	123.67±11.38	120.00±9.38	**119.0±9.22**	**0.63**
8	**AMYLASE**	63.8±12.7	61.2±12.8	58.9±12.6	73.2±22.8	**0.002**
9	LIPASE	1±0.4	0.7±0.4	0.8±0.4	0.6±0.3	0.12
10	CHOLESTEROL	157.23±7.11	**178.06±5.85**	145.43±3.75	161.16±7.06	0.9
11	TRIGLYCERIDES	122.16±2.83	**142.65±2.89**	124.77±2.14	123.74±2.18	0.16
12	**SGOT**	33.1±7.1	32.13±5.8	31.39±6.9	**28.4±6.8**	**0.81**
13	**SGPT**	32.13±8.2	30.42±1.94	31.07±1.98	**27.6±7.7**	**0.73**
14	URIC ACID	4.3±0.9	3.9±0.8	4.1±0.6	4.0±1.0	0.57
15	**LDH**	107.23±6.88	98.61±2.64	103.68±6.18	**101.68±6.98**	**0.93**

Serum potassium was found to be stable up to T30d when the serum samples were separated from cells and stored in aliquots, whereas previous studies have demonstrated an increase in K+ after 24 h due to serum-cell contact at room temperature.[2,5] The increase in K+ after 24 h is most likely caused by malfunction of the Na+/K+ ATPase pump, resulting in diffusion of K+ from the erythrocytes driven by the intracellular–extracellular concentration gradient.[8]

Moreover, our results showed a clinically insignificant increase in **K+** level at T15d. However, no significant statistical or clinical differences were observed between the levels of the metabolites (Na+, K+, urea, creatinine, uric acid, total bilirubin and direct bilirubin) in fresh samples and in samples stored at −20°C for 7, 15, and 30 days. These results are in agreement with those of a previous study [9] reporting that total bilirubin, and direct bilirubin showed clinically equivalent levels, but that serum urea levels exhibited an appreciable increase in BUN values over time ;however, BUN instability, indicated by a substantial decrease in levels, has been reported for samples stored at −20°C.[10]

Consistent with a previous study ,[10] we did not detect any statistically or clinically significant change in creatinine levels. However, according to Boyanton and Blick, the increase in serum creatinine levels after 24 h is due to serum-cell contact at room temperature.[3] our results showed a serial decrease in serum **uric acid** concentration over time; however, these changes were neither statistically nor clinically significant.

Lipid

Total cholesterol and triglyceride concentrations were stable up to T30d, in agreement with the findings of Kamal Kachhawa and et al. Cuhadar et al. [9,11]

Enzymes

A decrease in the concentration of serum **ALP, ALT, AST,** and **LDH** was observed; however, these changes were not statistically or clinically significant. Our findings regarding serum AST are consistent with those of Cuhadar et al. [9] In addition, according to Paltiel et al.,[11] AST activity remains stable for 10–15 freeze-thaw cycles following storage at −80°C. Significant statistical or clinical differences were observed between **serum amylase** levels in fresh samples and samples stored at −20°C for 7, 15, and 30 days; serum amylase levels decreased with prolonged storage. To the best of our knowledge, this is the second report regarding the effects of storage at low temperatures on serum amylase levels. Our findings regarding serum Amylase are consistent with those of Kamal Kachhawa and et al. Cuhadar et al.

CONCLUSION

Most of the metabolites as well as enzymes aside from serum amylase, showed adequate stability following up to 30 days storage at −20°C. These results also indicates whether deep freezing at −20°C can be useful tool for additional analyses at later time points as well as for research purposes, which require that samples be stored for longer periods until batch analysis can be conducted. This knowledge will help us to improve the **precision and accuracy** of current diagnostic strategies.

Study Limitation

- 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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