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Original Research Article

Does Extended Serum Sample Storage Impact Laboratory Results?

Balaji Pillai[1], Ajeet Kumar Khilnani[1], Dinesh Sharma[2]

Departments of [1]Otorhinolaryngology and [2]Biochemistry, Gujarat Adani Institute of Medical Sciences, Bhuj, Kachchh, Gujarat

*Correspondence: Dr Dinesh Sharma (dksharma0305@gmail.com)

ABSTRACT

Background: Prolonged storage of samples can result in changes to routine biochemical parameters.

Aim: This study aimed to examine the biochemical changes in serum samples stored at –20°C for extended periods (72 hours), focusing on various routine biochemical parameters.

Results: Significant changes were noted in serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and potassium levels after 72 hours of storage, with further deterioration observed over 3 months. The remaining parameters showed no significant alterations.

Conclusion: Prolonged sample storage results in changes in analyte concentrations in serum. Serum glucose, AST, ALT, creatinine, and potassium levels should be measured within 72 hours if extended storage is necessary. All parameters should ideally be analyzed within 24 hours to prevent misinterpretation of results and ensure optimal patient care.

Keywords: AST, ALT, Creatinine, Biochemical parameters

INTRODUCTION

Laboratory investigation play a vital role in diagnosing and monitoring the treatment of various diseases. Storage duration is a key factor that can influence the reported values of analytes. Blood sample storage can result in alterations to both biochemical and physical properties due to storage conditions. These alterations in the sample are known as storage lesions.[1] The primary cause of storage lesions is hemolysis, which can impact a sample in multiple ways. Hemolysis may affect a blood sample through mechanisms such as erythrocyte rupture and the release of intracellular contents into the serum, hemodilution, or the direct influence of hemoglobin concentration on specific analyte levels.[2] For routine assays in a clinical laboratory, serum is typically used as the sample. To accurately detect pathological changes in patients, it is crucial to minimize storage lesions to levels where they do not affect the clinical interpretation of results. Standard guidelines for blood sample handling recommend that plasma or serum be separated within 20 to 30 minutes, or as soon as possible after clot formation, to prevent clot-induced alterations in the concentration of serum analytes.[3] Prolonged contact between plasma/serum with cell is common reason of inaccurate test results. Therefore, plasma and serum should ideally be segregated from cells as rapidly as

possible to prevent the ongoing cellular components metabolism.[4,5] Many investigators have examined the changes in various analytes due to storage, but the results remain controversial. Therefore, the present study was designed to assess the impact of storage time on laboratory results for glucose, total protein, albumin, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, total cholesterol, urea, uric acid, potassium, sodium, and chloride in pooled serum over different time intervals. This study aimed to identify the quantitative changes and find the optimal storage duration for serum samples, and the clinical significance of these alterations.

MATERIALS AND METHODS

The study was conducted in the Dept. of Biochemistry at Gujarat Adani Institute of Medical Sciences and GK General Hospital, Bhuj, Gujarat, India. Sample anonymization was performed, and only samples from healthy male volunteers were included. After ensuring proper aseptic techniques, 4 mL of blood was drawn from 10 healthy adult males, aged between 20 and 50 years, into red-top vacutainers. The participants were instructed to fast overnight prior to the blood collection. The samples were allowed to clot at room temp. for 20 min & centrifuged at 3,500 rpm for 7 min. To prepare pooled serum, samples from all 10 participants were combined in a sterile container, thoroughly mixed, and divided into 1 mL aliquots, which were stored at –20°C. Initial analysis (zero time) was conducted immediately on the separated serum for 14 analytes. Subsequently, the aliquots were stored at –20°C, and analyses were performed at intervals of 0, 24 hours, 48 hours, 72 hours, 1 week, 15 days, and 1, 2, and 3 months. For each analysis, a single aliquot was used. The analytes were estimated using the following methods: glucose (GOD method), total protein (Biuret Method), Albumin (BCG-bromcresol green dye method), Total bilirubin and direct bilirubin (Modified Jendrassik), and AST (IFCC method), ALT (IFCC method), Alkaline phosphatase (IFCC method), urea (urease method), creatinine (modified Jaffe's method), uric acid (Enzymatic uricase method), and Total cholesterol (CHOD-POD method) was using Vitros 7600 fully automated analyzer. Electrolytes Na^+, K^+, Cl^- were estimated by using Vitros 7600 analyzer based on direct ion selective electrode method. Samples exhibiting visible hemolysis and icteric samples were keep out from the study. The data were assayed by using standard statistic method and the results were showed as percentage increases or decreases after analysis at different time interval.

RESULTS

A pooled serum, prepared from the blood of 10 males subject, was analyzed. The average age of the subject was 30 ± 5 year. The various biochemical parameters analyzed are presented in Table-1.

Table-1: Assay Values for Pooled Serum at 0 hour, 24 hours, and 48 hours

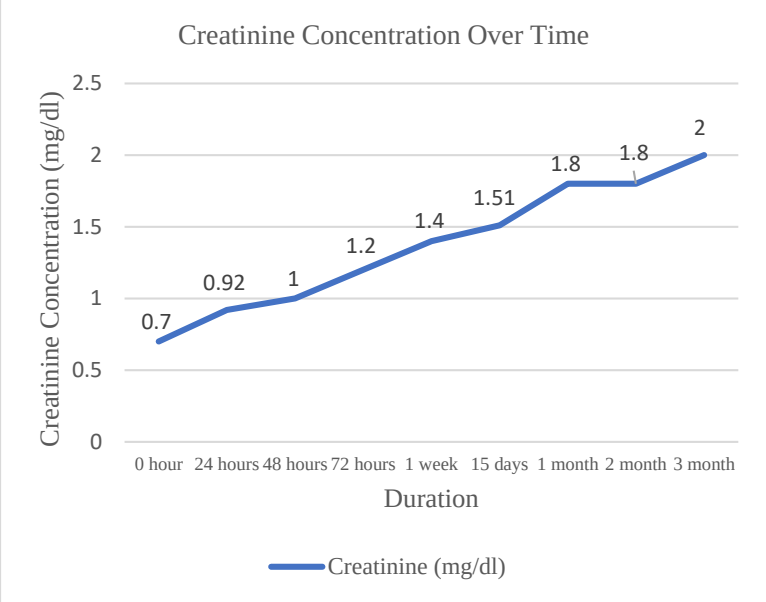
Values obtained (average of 10 samples)				
Analyte	**0 -hour**	**24 hours**	**48 hours**	**Reference intervals**
Glucose (mg/dL)	111.8	110.5	99.8	70–100
Total Protein (Gm/dL)	7.25	7.25	7.26	6.0-8.0
Albumin (Gm/dL)	3.36	3.38	3.35	3.5-5.0
Total Bilirubin (mg/dL)	0.80	0.81	0.82	0.2–1
Direct Bilirubin (mg/dL)	0.2	0.3	0.25	0.1–0.4
Aspartate aminotransferase (IU/L)	32	31	32	0-40
Alanine aminotransferase (U/L)	28	29	30.1	0-40
Alkaline phosphatase (U/L)	101.2	101.8	102.2	35–129
Urea (mg/dl)	36.1	36.2	36.4	20–40
Creatinine (mg/dL)	0.70	0.92	1.0	0.7–1.4
Uric Acid (mg/dl)	4.6	4.70	4.72	3.5–7
Total Cholesterol (mg/dl)	160	163.5	162.8	150–200
Sodium (Na^+) (mmol/L)	142	143	143	135–145
Potassium (K^+) (mmol/L)	4.2	4.6	5.2	3.5–5
Chloride (Cl^-) (mmol/L)	101	103	104.5	95–105

Table-1 indicates that no significant changes were observed in the conc. of analytes within the first 48 hours. The analytes were subsequently re-evaluated at interval of 72 hours, 1 week, 15 days, 1 month, 2 months, and 3 months from the initial 0-hour point, as shown in Graphs 1 & 2 and Table-2.

Table-2: Alterations in analyte concentration in pooled serum over time when stored at –20°C (average of 10 samples)

Analytes	72 hour	1 week	15 days	1 month	2 months	3 months
Glucose (mg/dl)	92.12	87.4	84.3	85.2	71.4	69.70
Total Protein (Gm/dL)	7.27	7.24	7.24	7.20	7.20	7.15
Albumin (Gm/dL)	3.34	3.32	3.32	3.25	3.25	3.20
Total Bilirubin (mg/dL)	0.81	0.70	0.75	0.76	0.72	0.72
Direct Bilirubin (mg/dL)	0.28	0.26	0.24	0.25	0.24	0.23
Aspartate aminotransferase (U/L)	32.4	42.2	44.6	46.8	55.3	61.0
Alanine aminotransferase (U/L)	30	38.1	43.5	47.7	50.3	60.4
Alkaline phosphatase (U/L)	105.2	109.2	112.4	112	115	118
Urea (mg/dL)	35	36	36.6	36.9	37	38
Creatinine (mg/dl)	1.21	1.40	1.51	1.8	1.8	2
Uric acid (mg/dl)	4.50	4.60	4.50	4.48	4.40	4.12
Total cholesterol (mg/dL)	152.1	160.8	160.8	159.2	157.6	155.6
Sodium (Na^+) (mmol/L)	140	142	140	139	137	136
Potassium (K^+) (mmol/L)	6.5	10.3	17.2	23.5	30.2	35.4
Chloride (Cl^-) (mmol/L)	104.6	105.4	107.1	108.5	111.4	113.1

In this study, no statistic significant changes were seen in the analyte levels up to 48 hours when samples were stored at –20°C. However, significant changes were detected in and after 72 hours in certain parameters. As shown in Table-2 and Graph-2, significant alterations were noted in Glucose, Aspartate aminotransferase, Alanine aminotransferase, K^+ levels & creatinine concentration. Glucose concentration decreased by approximately 17.62% within 72 hours and by 37.64% after 3 months. AST levels increased by 1.2% from the initial value at 72 hours, with a further rise of 90.63% at the end of three months. Alanine aminotransferase conc. increased by 7.14% after 72 hours & by 115.71% after 3 months. Potassium conc. increased by 57.14% at 72 hours and surged by a significant 742.86% at the 3-month mark shown in Table-2 and Graph-2. Creatinine conc. rose by 71.43% at 72 hours & by 185.71% at 3 months. In summary, glucose conc. decreased by 17.62% within 72 hours of storage shown in Graph-1. Creatinine levels increased by 71.43% after 72 hours & by 185.7% after 3 months. This significant rise is attributed to the interference of pseudocreatinines (pc), as demonstrated by Heins et al.[13,14] Such a substantial increase cannot be solely explained by hemoconcentration.

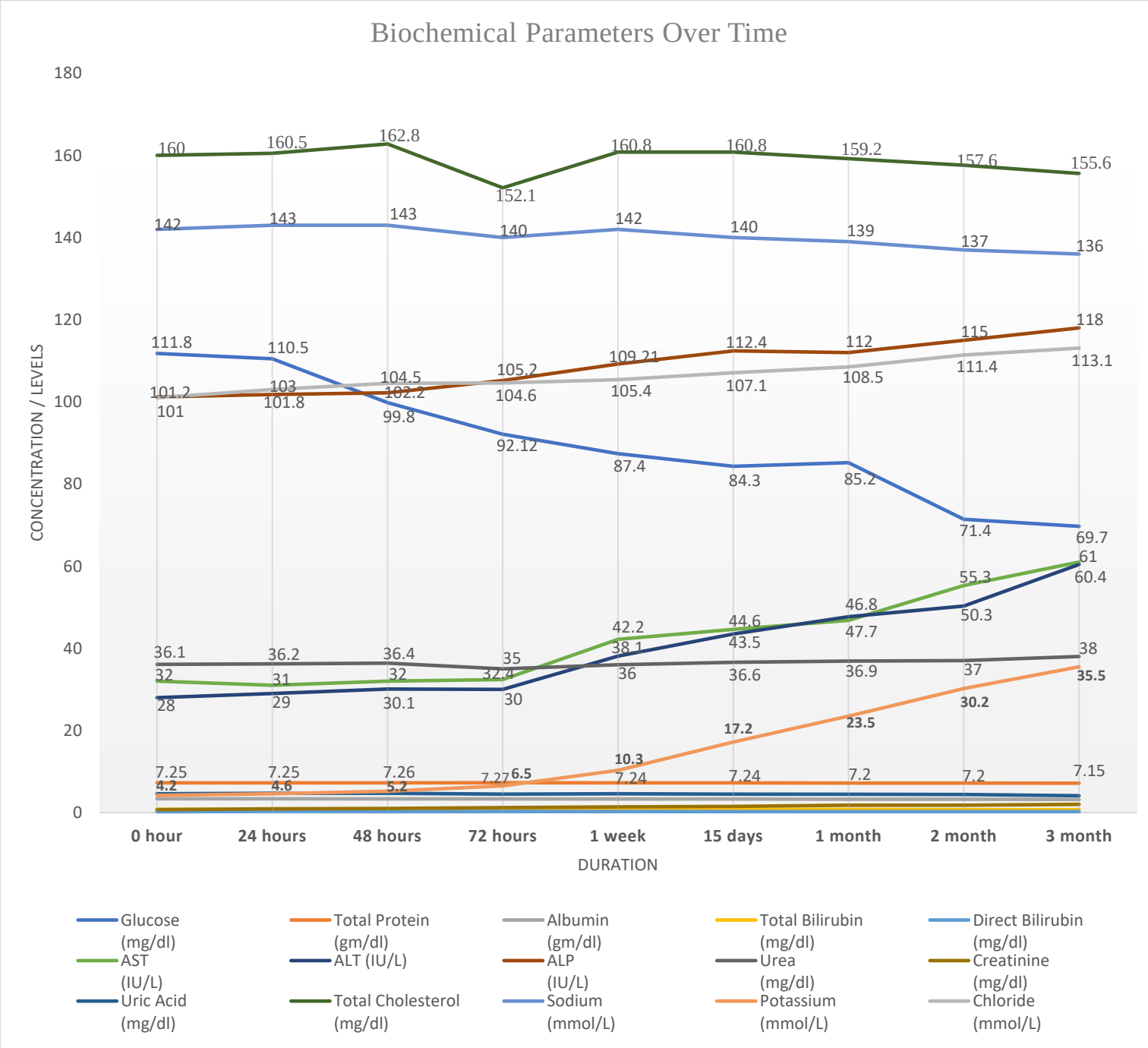


Graph-1: Alterations in creatinine concentration in pooled sera over different time interval (average of 10 samples)

Potassium concentration showed a gradual increase after 24 hours, with a significant 57.14% rise seen after 72 hours. This increase in K^+ level during storage of centrifuged tube is attributed to the presence of a small number of RBCs on the surface of (PSG) polyester

separation gel. The inhibition of the sodium-potassium pump contributes to hyperkalemia & hyponatremia, as spotted in this study.[15,16] Adias et al.[11] also reported hyperkalemia in their study but did not find significant changes in sodium levels, which aligns with the present findings.

Our findings align with those of previous studies conducted on serum samples.[3,4] The other constituents did not show any significant changes over times when the samples were promptly centrifuged upon receipt and stored under stringent conditions at –20°C.



Graph-2: Alterations in various biochemical parameters in pooled sera at different time intervals (average of 10 samples)

DISCUSSION

According to Jandl[6], glucose levels in serum decline over time, accompanied by a corresponding increase in concentration of lactate, with a theoretical net Glucose decrease to lactate production ratio of 1:2. High initial levels of steady-state glycolytic intermediates in RBCs at the collection time, may undergo glycolysis to produce lactate, further contributing to the reduction in glucose concentration[7]. In routine whole blood samples, glucose availability is limited, and over time, it is utilized by red blood cells (RBCs) for metabolism, as under physiological conditions. This process leads ATP depletion and a subsequent decrease in RBC viability[7]. The diminished ATP required for the ATPase pump in erythrocytes over time explains the observed reduction in glucose concentration, as shown in Table-2. Similar declines in plasma glucose concentration during storage were reported by Latham et al[8]. and Bailey and Bove.[9] Significant changes were seen in Aspartate aminotransferase and Alanine aminotransferase levels, likely due to hemolysis, as noted in other studies. Koseoglu et al.[10] study the effect of hemolysis on routine biochemical parameters, indicating that hemolysis interference influenced AST levels even at undetectable hemolysis (plasma hemoglobin <0.5 g/L). In contrast, ALT levels remained unaffected until severe hemolysis (hemoglobin: 2.5–4.5 g/L). RBCs contain AST concentrations approximately 20 times higher than plasma, making AST particularly susceptible to even mild hemolysis. In this study, a 51% increase in AST levels was observed from 0-day to 15 days. The unequal correlation between AST and Alanine aminotransferase is consistent with the higher AST concentrations found in RBCs delivered during hemolysis. However, no specific cause for this discrepancy in serum samples has been documented.[11] Increased serum ALT and AST levels may also result from specimen storage, as both enzymes can become activated during storage, artifactually increasing their activities.[12] Additionally, elevated lactate level after 72 hours could interfere with the methodology, further contributing to the observed changes.

Limitations

- Pseudo-creatinine was not measured in this study due to the unavailability of facilities, which might have contributed to the observed changes in creatinine value after storage.
- Long-term storage may have caused slight variations in sample volume, potentially leading to minor changes in analyte concentrations, although these changes are unlikely to be noteworthy in the current context.

CONCLUSIONS

Our study finds that even when samples are promptly centrifuged upon receipt in the laboratory & stored at –20°C, there is a progressive deterioration in glucose, Aspartate aminotransferase, Alanine aminotransferase, creatinine & potassium levels after 72 hours. To ensure accurate laboratory results and avoid misinterpretation, it is recommended that all parameters be analyzed as soon as the sample is received.

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