

Does Prolonged Storage of Serum Samples alter the Lab Results?

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ABSTRACT

Background: Sample storage for prolonged periods can lead to alterations in routine biochemical parameters.

Aim: This study was undertaken to observe the biochemical changes on serum samples when they are stored for prolonged periods at -20°C on different routine biochemical parameters.

Results: Significant changes were observed in serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and potassium levels after 72 hours of storage, which further deteriorate in 3 months. Rest of the parameters did not show any significant alteration.

Conclusion: Prolonged storage of samples lead to alterations in the analyte concentration in serum. Serum glucose, AST, ALT, creatinine, and potassium levels should be estimated within 72 hours if prolonged storage is unavoidable. In conclusion, all the parameters should be assayed within 24 hours to avoid misinterpretation of results and better patient care.

Keywords: Alanine transaminase, Aspartate transaminase, Biochemical parameters, Creatinine.

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INTRODUCTION

Laboratory tests aid in the diagnosis as well as in monitoring treatment of various diseases. Storage time is an important factor that can affect the reported value of an analyte. Storage of blood sample may lead to change in biochemical as well as physical properties because of storage conditions. These changes in the sample are referred to as storage lesions.¹ The most common cause of storage lesions is hemolysis, which can affect a sample

in a number of ways. Various mechanisms by which hemolysis can affect a blood sample may be erythrocyte rupture and release of intracellular contents into the serum, hemodilution or hemoglobin concentration directly affecting the particular analyte concentration.² For routine assays in a clinical laboratory, serum is the sample. To detect real pathological changes in patients, such storage lesions must be reduced to acceptable levels at which they cause no impact on clinical interpretation of the results. Standard guidelines for blood sample handling state that plasma or serum should be separated within 20 to 30 minutes or from the cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analytes.³ Because prolonged contact of plasma or serum with cells is a common cause of spurious test results, plasma and serum should ideally be separated from cells as quickly as possible to prevent ongoing metabolism of cellular constituents.^{4,5} Many investigators have studied the related changes in some analytes, but the results are controversial. Thus, the present study was designed to determine the effect of storage time on the laboratory results of glucose, total protein, albumin, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, uric acid, total cholesterol, sodium, potassium, and chloride in pooled sera at different time intervals. In this study, we tried to find out the quantitative alterations and the useful length of stored serum in different time on the laboratory results and the clinical significance of such alterations.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, National Institute of Tuberculosis and Respiratory Diseases, New Delhi, India. Sample anonymization was done and only the samples from the healthy volunteer male subjects in the laboratory were included in the study. After taking all the necessary aseptic precautions, 4 mL of blood was collected from healthy adult males of 20 to 50 years old (in total 10 adult males) in a red top vacutainer. The adult males were instructed to fast overnight before blood collection was completed. Samples were allowed to clot at room temperature for 20 minutes, and then centrifuged at 3,500 rpm for 7 minutes. To get the pooled sera, serum was collected from all the 10 samples

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Table 1: Assay values in pooled serum immediately after collection 0 hour, 24 hours and 48 hours with reference intervals

Analyte	Values obtained			Reference intervals
	0 hour	24 hours	48 hours	
Glucose (mg/dL)	112.8	111.5	98.8	70–110
Total protein (gm/dL)	7.30	7.27	7.25	4.5–7.5
Albumin (gm/dL)	3.38	3.37	3.34	3.5–4.5
Total bilirubin (mg/dL)	0.81	0.81	0.81	0.2–1
Direct bilirubin (mg/dL)	0.3	0.3	0.29	0.1–0.4
Aspartate aminotransferase (U/L)	30	31.2	32.1	0–40
Alanine aminotransferase (U/L)	28.9	29.4	30.2	0–40
Alkaline phosphatase (U/L)	101.3	101.7	102.3	35–129
Urea (mg/dL)	36.2	36.3	36.4	20–40
Creatinine (mg/dL)	0.72	0.93	1.0	0.7–1.4
Uric acid (mg/dL)	4.8	4.76	4.73	3.5–7
Total cholesterol (mg/dL)	165	163.1	162.5	150–200
Sodium (mmol/L)	143	143	142	136–145
Potassium (mmol/L)	4.4	4.7	5.1	3.5–5
Chloride (mmol/L)	102	103.7	104.1	96–106

in sterile container, mixed, and aliquots of 1 mL each were prepared and stored at -20°C . Analysis was performed on the separated sera for 10 analytes without delay (zero time). Furthermore, each sample was stored in the refrigerator at -20°C and estimations were performed at 0, 24 hours, 48 hours, 72 hours, 1 week, 15 days, 1, 2, and 3 months. Only one aliquot was used each time the analysis was performed.

Estimation of glucose (glucose oxidase method), total protein (biuret reaction), albumin (bromocresol green dye-binding method), total bilirubin and direct bilirubin (modified Jendrassik), AST [International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate activation], ALT (IFCC method without pyridoxal phosphate activation), alkaline phosphatase (ALP) (IFCC method with aminomethylpropanol buffer), urea (uricase method), creatinine (modified Jaffe's method), uric acid (uricase peroxidase), and total cholesterol (cholesterol oxidase) was using ROCHE Hitachi H 902 fully automated analyzer. Electrolytes (Na, K, Cl) were analyzed using ROCHE electrolyte analyzer 9180 based on direct ion-selective electrode method.

The samples showing visible hemolysis and the icteric samples were excluded from the study. Data were analyzed using standard statistical methods and the results were expressed in percentage increase or decrease after analyzing at different intervals.

RESULTS AND DISCUSSION

A pooled serum made from the serum of 10 male subjects was analyzed. Mean age of the subjects was 30 ± 5 years. The various biochemical parameters analyzed are shown in Table 1.

Table 1 shows no statistically significant changes in the concentration of analytes till 48 hours. All the analytes were reassessed at intervals of 72 hours, 1 week, 15 days, 1, 2, and 3 months from the 0 hours as seen in Graph 1 and Table 2.

In this study, we could not find any statistically significant changes in the levels of the analytes till 48 hours when the samples are stored at -20°C , but significant changes are observed after 72 hours in some parameters.

As seen from Table 2, significant changes in concentration were seen only in glucose, AST, ALT, creatinine, and potassium. Glucose concentration reduced by about 18.3% by the end of 72 hours, and it is decreased by 38.2% by the end of 3 months. The AST concentration increased by 11.3% from the 0 hour value by the end of 72 hours and it increased by 105% by the end of 3 months. The ALT concentration increased by 10.7% from the 0 hour value by the end of 72 hours and it increased by 102% by the

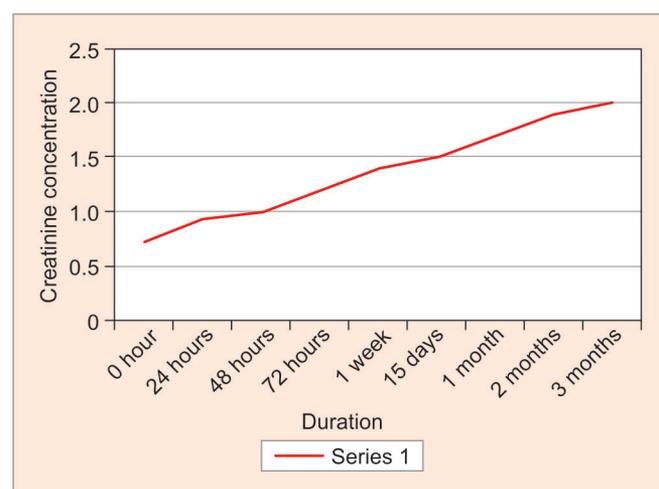
**Graph 1:** Changes in creatinine concentration in pooled serum over time

Table 2: Changes in analyte concentration in pooled serum over time when stored at -20°C

Analyte	72 hours	1 week	15 days	1 month	2 months	3 months
Glucose (mg/dL)	92.1	86.4	84.3	80.2	75.4	69.7
Total protein (gm/dL)	7.28	7.25	7.24	7.22	7.20	7.16
Albumin (gm/dL)	3.35	3.33	3.30	3.26	3.25	3.23
Total bilirubin (mg/dL)	0.80	0.78	0.77	0.75	0.72	0.7
Direct bilirubin (mg/dL)	0.29	0.28	0.26	0.25	0.24	0.21
Aspartate aminotransferase (U/L)	33.4	42.2	44.6	47.8	55.3	61.5
Alanine aminotransferase (U/L)	32	38.1	43.5	48.7	52.3	58.4
Alkaline phosphatase (U/L)	103.3	107.3	110.4	112.2	115.4	119
Urea (mg/dL)	36.3	36.5	36.6	36.9	37.1	38.2
Creatinine (mg/dL)	1.2	1.4	1.5	1.7	1.9	2
Uric acid (mg/dL)	4.68	4.64	4.52	4.48	4.35	4.1
Total cholesterol (mg/dL)	162.1	161.8	160.4	158.2	156.3	155.8
Sodium (mmol/L)	142	140	139	138	137	135
Potassium (mmol/L)	6.6	10.3	17.3	23.4	30.1	35.4
Chloride (mmol/L)	104.7	105.5	107	108.2	111.5	113.2

end of 3 months. Creatinine concentration increased by 71.4% from the 0 hour value by the end of 72 hours and it increased by 185.7% by the end of 3 months. Potassium concentration increased by 50% from the 0 hour value by the end of 72 hours and it increased by a massive 704% by the end of 3 months.

Glucose concentrations decreased by 18.3% by 72 hours. According to Jandl,⁶ glucose concentration in serum decreases with time, with consequent increase in lactate concentration. The ratio of net glucose loss to net lactate production is theoretically 1:2. Additionally, high concentration of initial, steady-state glycolytic intermediates within the erythrocytes at the time of specimen collection that subsequently undergo glycolysis to yield lactate which further lead to decreased glucose concentration.⁷ In a routine whole blood sample, glucose concentration will be limited and with time glucose is utilized by the red blood cell (RBC) for their metabolism as in physiological conditions which leads to consequent adenosine triphosphate (ATP) depletion and decrease in red cell viability.⁷ So, energy required for operating ATPase pump in RBCs decreases with time which can explain the decrease in glucose concentration in the sample with time, as seen in Table 2. Latham et al⁸ and Bailey and Bove⁹ also observed decline in concentration of plasma glucose and with storage.

Significant changes were observed in AST and ALT levels, which can be because of hemolysis as observed in other studies. Koseoglu et al¹⁰ studied the effects of hemolysis interferences on routine biochemistry parameters. Hemolysis interference affected AST at undetectable hemolysis by visual inspection (plasma hemoglobin <0.5 gm/L). The ALT concentrations were not interfered up to severely hemolyzed levels (hemoglobin: 2.5–4.5 gm/L). The RBCs contain 20-fold as high concentration of AST

as plasma, so even mild hemolysis produces significant alterations in AST. We found 51% increase from day 0 to day 21 in AST levels. The disproportionate correlation with AST, and not ALT, is consistent with higher concentrations of AST than ALT in RBCs released during hemolysis, but in serum samples no cause has been documented.¹¹ Increased serum concentration of ALT and AST in serum may also be attributed to the storage of specimens, which activate both enzymes as early as storage has the effect of artifactually increasing the activities of these enzymes.¹² Increased lactate concentration after 72 hours may also have interfered with the methodology.

Creatinine increased by 71.4% after 72 hours and by 185% after 3 months (Graph 1), which was attributable to interference of pseudocreatinines as shown by Heins et al.^{13,14} Such massive increase cannot be explained just by hemoconcentration.

Potassium concentration though showed increase after 24 hours, significant increase of 50% was seen only after 72 hours. This increase in potassium concentration after storage of centrifuged tubes is caused by the small number of erythrocytes on the surface of the polyester separation gel. Inhibition of sodium pump leads to hyperkalemia and hyponatremia as observed in the present study.^{15,16} Adias et al¹¹ also observed hyperkalemia in their study but they did not find any significant change in Na^+ , which goes in line with the present study. Our findings were consistent with previous studies in serum.^{3,4}

Other analytes did not show any significant alterations over a period of time when the sample is centrifuged as soon as it is received and stored under stringent storage conditions of -20°C .

CONCLUSION

Our study confirms that even when serum samples are centrifuged as soon as samples are received in the clinical

laboratory and stored at -20°C , there will be progressive deterioration of glucose, AST, ALT, creatinine, and potassium levels after 72 hours. In conclusion, our study will help to assess which of the analytes may be assayed till what time period when prolonged storage occurs inadvertently or is unavoidable. Furthermore, the parameters should be assayed as soon as sample is received to get valid laboratory results and prevent the misinterpretation of results.

LIMITATIONS

- Pseudocreatinines were not estimated which might have interfered with the final creatinine values after storage due to lack of facilities available for the same.
- Long-term storage could have led to the changes in sample volume that could have led to small changes in concentration but that will not be significant in the present context.

REFERENCES

1. Verma M, Dahiya K, Malik D, Sehgal PK, Devi R, Soni A, Ghalaut VS. Effect of blood storage on complete biochemistry. *J Blood Disord Transfus* 2015;6:329.
2. Sonntag O. Haemolysis as an interference factor in clinical chemistry. *J Clin Chem Clin Biochem* 1986 Feb;24(2):127-139.
3. Brinc D, Chan MK, Venner AA, Pasic MD, Colatonia D, Kyriakopolou L, Adeli K. Long-term stability of biochemical markers in pediatric serum specimens stored at -80°C : a CALIPER substudy. *Clin Biochem* 2012 Jul;45(10-11):816-826.
4. Young DS, Bermes EW. Specimen collection and processing: sources of biological variation. In: Burtis CA, Ash Wood ER, editors. *Tietz textbook of clinical chemistry*. Philadelphia (PA): WB Saunders Company; 1999. p. 42-72.
5. Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. *Clin Chem* 1998 Jun;44(6 Pt 1):1325-1333.
6. Jandl JH. Physiology of red cells. In: *Blood: a textbook of hematology*. 2nd ed. Boston: Little Brown and Company; 1996. p. 157-177.
7. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. *Clin Chem* 1988 Oct;34(10):2111-2114.
8. Latham JT Jr, Bove JR, Weirich FL. Chemical and hematologic changes in stored CPDA-1 blood. *Transfusion* 1982 Mar-Apr;22(2):158-159.
9. Bailey DN, Bove JR. Chemical and hematological changes in stored CPD blood. *Transfusion* 1975 May-Jun;15(3):244-249.
10. Koseoglu M, Hur A, Atay A, Cuhadar S. Effects of hemolysis interference on routine biochemistry parameters. *Biochem Med* 2011;21(1):79-85.
11. Adias TC, Moore-Igwe B, Jeremiah ZA. Storage related haematological and biochemical changes of CPDA-1 whole blood in a resource limited setting. *J Blood Disorders Transf* 2012;3:124.
12. Ono T, Kitaguchi K, Takehara M, Shiiba M, Hayami K. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. *Clin Chem* 1981 Jan;27(1):35-38.
13. Nsiah K, Dzogbefia VP, Ansong D, Akoto AO, Boateng H, Ocloo D. Pattern of AST and ALT changes in relation to hemolysis in sickle cell disease. *Clin Med Insights Blood Disord* 2011;4:1-9.
14. 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 Apr;33(4):231-238.
15. Donnelly JG, Soldin SJ, Nealon DA, Hicks JM. Stability of twenty-five analytes in human serum at 22 degree C, 4 degrees C and -20 degrees C. *Pediatr Pathol Lab Med* 1995 Nov-Dec;15(6):869-874.
16. Boyanton BL Jr, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. *Clin Chem* 2002 Dec;48(12):2242-2247.