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Stability of Selected Biochemical Analytes in Plasma Samples Stored under Different Time and Temperature Conditions

Perez Quartey*, Quartey Perez, Orraca-Tetteh James and Seglah Richard Yawo

Department of Medical Laboratory Technology, Garden City University College, Kumasi, Ashanti, Ghana

***Corresponding author:** Quartey P, Garden City University College Kumasi, Ashanti Ghana; E-mail: quartey.perez@gcuc.edu.gh

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Abstract

Background: Though serum has traditionally been the main type of sample for biochemical tests, some laboratories are increasingly using plasma now due to factors such as inadvertent clogging of auto-analyzer probes by fibrin clot in serum and delay in sample testing due to time required for blood clotting. Many previous studies on stability of biochemical analytes have mainly focused on serum with limited works on plasma.

Aim: We studied the stability of commonly used clinical biochemical analytes in human plasma including the comparative effects of storage time (0, 7, 14, 21 days) and temperature (room temperature, 4°C-8°C refrigeration temperature and -60°C) on blood separated plasma.

Methodology: Plasma samples were obtained from 6 healthy adults who are not on any medication. Urea, creatinine, sodium, potassium, total bilirubin, direct bilirubin and total protein measurements were done on freshly separated plasma sample immediately as baseline measurement. Three aliquots each were stored at room temperature on the laboratory bench, refrigerator, and -60°C freezer. Measurements were done on the stored samples on days 7, 14 and 21 and then comparatively analyzed for stability.

Results: All analytes were fairly stable in samples stored at -60°C during the study period. However, there were variations in urea, creatinine, sodium and total protein in samples stored at room temperature and refrigerator temperature. Statistically significant increases were observed in urea and creatinine with reduction in sodium after 7 days in room temperature stored samples. Similar changes were also seen in refrigerator samples after 14 days. Clinically significant changes were seen in creatinine, sodium and urea after 7 days, 14 days and 21 days respectively. Statistically significant reduction in total protein was observed in both room temperature and refrigerator samples after 14 days. Bilirubin and potassium were fairly stable in all samples during the study period.

Conclusion: Biochemical analytes are desirably stable after 3 weeks of storage in plasma separated samples at -60°C. However, there are considerable variations in stability of biochemical analytes in plasma separated samples during storage at room and refrigerator temperatures and these have to be taken into consideration in conditions of prolonged delays in analysis of samples. Additionally, the observed variations are worth investigating, in the light of inconsistencies with previously reported works, to further identify factors and mechanisms that affect the stability of biochemical analytes in plasma separated samples.

Keywords: Plasma separated samples; Analytes; Stability; Storage time; Temperature

Introduction

In the clinical laboratory setting, several factors may demand the storage of blood samples including number of samples, availability of reagents and breakdown of equipment. Additionally, research work may also require the need for storage of samples for longer periods. Therefore a general source of concern in clinical laboratory testing is maintenance of the stability of analytes in blood and blood components during sample storage. Samples are usually stored in refrigeration condition (4°C–8°C) or in a deep freezer (below–20°C). Sample handling, storage time and temperature are therefore considered important pre-analytical factors that may affect consistency or variations in test results in the clinical biochemistry laboratory.

Though serum has traditionally been the main sample type for biochemical tests, some laboratories are increasingly using plasma now due to factors such as inadvertent clogging of auto analyzer probes by fibrin clot in serum and delay in sample testing due to time required for blood clotting.

Many previous studies on stability of biochemical analytes have mainly focused on serum with limited reported works on plasma samples. The effects of storage on the stability of analytes in plasma with the use of a plasma separator tubes have been reported [1,2]. The effects of serum-clot time on the stability of analytes have been reported in serum immediately separated and after prolonged contact with cells [3,4,5]. Some studies have also been done on the effects of freeze-thaw cycles on stored serum [6] and the stability of biochemical analytes in serum stored at predefined temperature conditions for varying lengths of time [7]. Some investigators have studied the stability of analytes in serum [8] and plasma [2] stored at room

temperature and refrigerator for 72 h. Some of these previous studies have given inconsistent results.

The current study examined the stability of selected commonly used clinical biochemical analytes in human plasma including the comparative effects of storage time (0, 7, 14, 21 days) and temperature (room temperature, 4°C-8°C refrigeration and -60°C) on blood-separated plasma.

Materials and Methods

Study design

The study was approved by the Institutional Research Committee and informed consent was obtained from volunteer participants after the study was explained to them. All procedures were conducted in accordance with the guidelines of the Helsinki declaration on human experimentation.

Sample collection and processing

Venous blood (4.5 mL) was collected from each of 6 healthy volunteers who were not fasting or on any medication, using standard procedures. Blood samples were dispensed into Vacutainer Plasma Separator Tubes containing lithium heparin (PST; Becton Dickinson). All the tubes were allowed to stand at room temperature for 30 min followed by centrifugation at 3500 rpm for 10 min. Samples were visually checked for hemolysis and interference. Plasma samples were pooled into a plain tube and aliquot into 1.5 mL Eppendorf tubes (Merck) and wrapped with aluminium foil (Ever pack). Urea, creatinine, sodium, potassium, total bilirubin, direct bilirubin and total protein measurements were done on one freshly separated aliquot sample immediately as baseline measurement (T_0) as shown in Table 1.

Analysis	Assay Methods
Urea	Urease-GLDH method
Creatinine	Modified Jaffe method
Sodium	ISE
Potassium	ISE
Total protein	Biuret method
Total bilirubin	Diazo method
Direct bilirubin	Direct bilirubin
Key: GLDH: Glutamate dehydrogenase; ISE: Ion-selective electrode	

Table 1: Biochemical measurements.

Three aliquots each were stored at room temperature on the laboratory bench, refrigerator, and -60°C freezer. The biochemical measurements were done on each of the stored samples on day 7 (W1), 14 (W2) and 21 (W3). Separate stored aliquots were used for each measurement period to avoid the effects or repeated freeze-thawing. At each measurement point, refrigerator and frozen samples were first brought to room temperature and the contents of all tubes were remixed before testing. All measurements were done in triplicates and the mean value was taken as the concentration of the analyte at that time-point of testing. The results were then comparatively analyzed for stability with the mean value of the baseline measurement as the reference point. All measurements were performed using Min dray BS 300 auto analyzer.

Analysis

The value for each analyte at each time point was determined as the mean value from three replicate measurements. Time-dependent changes in stored plasma analytes were determined by repeated measures ANOVA with statistical significance at p-value <0.05. Clinically significant changes were determined using the Significant Change Limit (SCL) approach as previously described [9]. SCL was defined as initial value ± 3.0 Usual Standard Deviation (USD) with the calculated mean for each analyte in the freshly separated plasma (T_0) representing the initial value. The USD was obtained by averaging the standard deviation of the quality control data of the previous 3 months for each analyte. The quality-control reference sample whose target mean most closely match the T_0 mean for each analyte was used to determine the USD [8]. Statistical analyses were performed with Graph Pad Prism version 5.0.

Results

The analysis results for the biochemical analytes measured in plasma samples under different storage conditions are shown in Table 2. All analytes were fairly stable in samples stored at -60°C throughout the study period. However, there were variations in urea, creatinine, sodium and total protein in samples stored at room temperature and refrigerator temperature. Statistically significant increases were observed in urea and creatinine with reductions in sodium and total protein after 7 days in room temperature and refrigerator samples. Clinically significant changes were seen in creatinine, sodium and urea after 7, 14 and 21 days respectively. Statistically significant reduction in total protein was observed in both room temperature and refrigerator samples after 14 days, though this change was not clinically significant. However, we did not observe statistically or clinically significant changes in bilirubin and potassium in all samples during the study period.

Mean concentrations with time of analysis							
Analyte	**T_0**	**W1**	**W2**	**W3**	**USD**	**SCL range**	**p-value**
Urea (mmol/L) RT Refrigerator -60°C	3.42	5.71 5.31 3.43	6.22 6.22 3.42	7.26[α] 7.49[α] 3.43	1.08	0.16-6.64	<0.001 <0.001 0.859
Creatinine (umol/L) RT Refrigerator -60°C	81.9	90.2α 83.3	91.8 84.1	96.4 84.6	1.11	78.6-85.2	<0.001 <0.001

		81.7	82.1	82.2			0.460
Sodium (mmol/L) RT Refrigerator -60°C	136.4	134.4 136.6 136.3	132.2α 134.2 136.2	132.4 134.8 136.3	1.14	132.6-39.4	<0.001 <0.001 0.095
Potassium (mmol/L) RT Refrigerator -60°C	4.14	4.16 4.16 4.15	4.16 4.15 4.14	4.15 4.15 4.16	0.09	3.87-4.41	0.058 0.079 0.058
Total bilirubin (umol/L) RT Refrigerator -60°C	5.92	5.91 5.91 5.91	5.89 5.90 5.91	5.89 5.91 5.93	0.08	5.68-6.16	0.117 0.162 0.182
Direct bilirubin (umol/L) RT Refrigerator -60°C	4.98	4.98 4.98 4.99	4.96 4.98 4.98	4.96 4.96 4.98	0.11	4.65-5.31	0.169 0.418 0.878
Total protein (g/L) RT Refrigerator -60°C	64.5	64.3 64.6 64.5	62.3 63.8 64.6	62.4 63.6 64.7	1.04	60.9-67.1	<0.001 <0.001 0.422

Key: RT: room temperature; T_0: mean baseline measurement in freshly separated plasma; W1: mean at storage day 7; W2: mean at storage day 14; W3: mean at storage day 21; α: Clinically significant change compared to T_0; SCL: significant change limit; USD: usual standard deviation.

Table 2: Statistical analysis of time and temperature dependent changes in biochemical analytes in stored plasma samples.

Discussion

The observed findings of this study indicate that almost all of the studied analytes were fairly stable in samples stored at -60°C during the study period, consistent with previous work by Cuhadar et al. [6] and Kachhawa et al. [7] although Vernekar and Jabannavar [10] reported instability in urea and creatinine in samples stored at -20°C. Some investigators have reported fair stability of many analytes in serum; Marjani [8] and plasma; Jinks et al. [2] stored at room and refrigerator temperature for 72 h. The sodium results in the room and refrigerator samples were at variance to those reported by Boyanton and Blick [1], Chu and MacLeod [3] and Zhang et al. [4] which showed increase in sodium after prolonged contact with cells. A significant time-dependent increase in urea observed in this study was consistent with others previously reported by Boyanton and Blick [1], Cuhadar et al. [6] and Vernekar and Jabannavar [10] although substantial decrease of about 15% in levels has been reported by Brinc et al. [11]. Significant increases in both serum urea and creatinine levels with time in samples stored at -20°C have been reported by Vernekar and Jabannavar [10]. This similar finding was however observed in only the samples stored at room and refrigerator temperatures. Interference with pseudocreatinines has previously been attributed to increase in creatinine upon storage by Heins et al. [12]. Mechanisms for such changes need to be explored further, in the context of concomitant decrease in total protein as observed in our study. However, Boyanton and Blick [1] and Cuhadar et al. [6] have reported increase in total protein in their works. Potassium levels have also been reported by Boyanton and Blick [1], Chu and MacLeod [3] and Heins et al. [12] to increase in serum and plasma after prolonged contact time with cells, possibly as a result of disruption of the Na+/K+/ATPase pump with diffusion of K+ from cells driven by the intracellular to extracellular concentration gradient. In this study however, We did not observe any significant changes in potassium in all sample conditions. Bilirubin levels in samples protected from light have been reported to be fairly stable by prevention of photo-degradation; Sofronescu et al. [13]. Amin and Ahlfors [14] have reported decreases both at -20°C and at -80°C and Cuhadar et al. [6] have also reported decreases as a result of repeated freeze-thaw cycles. We observed bilirubin to be fairly stable in all samples during the study with the sample handling and storage.

Conclusion

In conclusion, the study showed that commonly measured biochemical analytes are stable in immediately separated plasma samples following 21 days of storage at -60°C. However, there are considerable variations in stability of biochemical analytes in plasma separated samples during storage at room temperature and refrigerator temperature and these have to be taken into consideration in conditions of prolonged delays in analysis of samples. Sodium, urea, creatinine and total protein are the least stable, exhibiting considerable variations in stability in samples stored at room temperature and refrigerator. The term "*Instability*" or "*Deterioration*" of biochemical analytes in plasma during storage does not always suggest reduction in concentrations of the analytes as observed in sodium and total protein but, in some cases rather unexplained increases as in urea and creatinine. Considering the observation that all the analytes in the samples stored at -60°C were stable with variations in stability seen in only the room temperature and refrigerator samples, it suggests temperature-mediated mechanisms as causes of these changes. The observed variations however, are worth investigating, in the light of inconsistencies with previously reported works. Additionally, excluding the effect of cell contact, it is important to investigate and identify the defined biochemical mechanisms underlying the causes of these variations in stability in plasma separated samples during storage conditions.

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Author contribution

This work was produced as part of a research thesis in the Department of Medical Laboratory Technology.

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