



Stability of Clinical Chemistry and Hematological Analytes in Preserved Plasma and Blood Obtained from Wistar Rats

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Summary

Blood samples obtained from experimental animals often need preservation due to several technical constraints. The present study was aimed to determine the effect of storage temperature and time on the stability of analytes in whole blood and plasma samples obtained from Wistar rats. Aspartate amino transferase, alkaline phosphatase, cholesterol, triglycerides, creatinine, urea, glucose, total protein, total bilirubin, phosphorous, sodium, potassium and chloride did not show statistically significant changes in plasma preserved at -20°C up to 4 weeks. However, alanine aminotransferase decreased by 4th week and gamma glutamyl transferase showed variance at different time points, while a statistically significant decrease was noted in albumin and calcium levels from the first week. A marked increase in LDH activity was noted after storage for 2 and 4 weeks. No substantial changes in complete blood count were noted. However, an increase was recorded in mean corpuscular volume at 72 hr and mean corpuscular hemoglobin at 48 hr and 72 hr in blood stored at $4 \pm 1^{\circ}\text{C}$. To conclude, the preservation temperatures of $-20 \pm 2^{\circ}\text{C}$ for plasma up to 4 weeks and $4 \pm 1^{\circ}\text{C}$ up to 96 hr for whole blood in Wistar rats did not remarkably affect the stability of analytes except for LDH and may be considered for laboratory investigations if warranted.

Introduction

Primary investigations of biological fluids and tissues are routinely conducted to identify applications of diagnostic and therapeutic importance (Subramanian, 1995). In clinical pathology, analyses of plasma or serum samples are essential tools to ascertain the toxicological impact of a test chemical. However, the integrity of the findings not only depends on the analytical instrument and equipment utilized, but also on the pre-analytical steps of collection, handling and storage of obtained samples (Subramanian, 1995; Heins et al., 1995; Zhang et al., 1998). Blood samples obtained from experimental animals often necessitate preservation owing to various technical constraints leading to delay in analysis from sampling

time, thus subjecting samples to different period of storage (Cray et al., 2009). Although frozen serum or plasma is frequently used in retrospective studies, interpretation and clinical analysis depends upon numerous critical factors of which stability of analyte is the most crucial (Thoresen et al., 1995). Concerns include instability of plasma and serum analytes (Boyanton & Blick, 2002), and variance in stability of analytes in plasma or serum samples obtained from the same animal origin (Thoresen et al., 1995; Mohri et al., 2008; Rendle et al., 2009; Peng et al., 2010).

Usually, studies of clinical importance employing human samples report stability of biochemical and haematological analytes in serum or plasma samples for pathological explorations (Heins et al. 1995;

Zhang et al. 1998; Gulati et al. 2002; Boyanton & Blick, 2002; Clark et al. 2003; Yue et al. 2008). Previous reports of stability involving plasma or serum obtained from various animal species include pigs (Fonda et al., 1982), dogs (Thoresen et al. 1995; Medaille et al. 2006), camels (Marjani, 2008), birds (Hawkins et al., 2006), goats (Divya & Jayavardhanan, 2010), dairy cows (Ehsani et al., 2008). Some studies report the effect of delayed haematological analysis of up to 72 hr on blood collected from turkeys (Hadzimusic et al., 2010), effects of temperature and duration on blood from western grey kangaroos and biochemistry or hematology of horses (Hulme-Moir et al., 2006; Rendle et al., 2009; Sharif et al., 2010). Analytically acceptable and clinically negligible changes in hematological parameters in blood from monkeys, rabbits, rats and mice stored at 4 °C were observed in samples kept up to 72 hr (Ameri et al., 2011). Other studies have shown that differences in sampling methods, locations, restraining, anesthesia and invasiveness can affect the outcome of serum biochemistry analysis (Deckardt et al., 1997; Fernandez et al., 2010). However literature on the stability of analytes for clinical chemistry and hematology from laboratory rats is scanty except some performed on Sprague-Dawley and Wistar-Kyoto rats for reference values to determine variation in biochemical parameters of serum and plasma (Caisey & King, 1980; Leonard & Ruben, 1980; Cray et al., 2009; Peng et al., 2010).

It is important to determine the stability of various analytes in stored plasma and blood for extended duration from murine animals that are routinely used for laboratory investigations. The present study was therefore designed to determine the influence of storage time and temperature on the stability of clinical chemistry parameters for up to 4 weeks and hematological parameters for up to 96 hr in blood samples obtained from Wistar rats.

Materials and Methods

Animals

Healthy adult male Lrpp: Wistar rats aged about 8-10 weeks were obtained from the Research Animal Facility of Lupin Limited (Research Park), Pune, India. The animals were kept in polypropylene cages with paddy husk bedding at a temperature of 23 ± 2 °C, relative humidity of 50 ± 20 %, 12 hr light and dark cycle, provided with potable drinking water and

standard pelleted diet (Nutrilab, Bangalore, India) *ad libitum*. The use of animals was approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design and sampling

A total of 15 animals were employed in the study and distributed into three groups (5 males in each) for clinical chemistry, haematology and a group for sodium, potassium and chloride (electrolytes) analysis as it requires the largest sample volume. Blood samples (approximately 2 ml) were collected from retro-orbital plexus of overnight fasted animals using fine capillary tubes and under mild anaesthesia using diethyl ether. Samples for haematology were collected in di-potassium salt of EDTA (Fischer Scientific, India) solution (20 % w/v) formulated in the laboratory. Samples for clinical chemistry and electrolyte analysis were collected in heparin sodium (Biological E, India) solution (25 IU/ml). Plasma was separated using a centrifuge (Hermle, Germany) at 2000 g for 15 minutes at 8 °C, and aliquots (150-160 µl) were subjected to different periods of storage, whereas aliquots (250 µl) of whole blood were stored for hematology (WHO, 2002). The freezer (-20 ± 2°C) used for storage was monitored for temperature throughout the experiment by using a calibrated thermometer.

Clinical chemistry

Two aliquots of plasma were kept at room temperature (23 ± 2 °C) for 6 hr and four aliquots were stored at -20 °C ± 2 °C for 24 hr, 1 week, 2 week and 4 week analysis respectively. Concurrent quality controls were run on the analyzer throughout the study. For clinical chemistry analysis, a Selectra-E auto-analyzer (Vital Scientific, Netherlands) was used. Electrolyte analysis was performed by using an Easylyte Plus Na/K/Cl Analyzer (Medica Corporation, The Netherlands). The estimations performed on plasma samples were glucose (GLU), cholesterol (CHOL), triglycerides (TGL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin (T.BIL), albumin (ALB), total protein (TP), urea (UREA), blood urea nitrogen (BUN), creatinine (CREAT), calcium (Ca), phosphorous (PHOS), chloride (Cl), sodium (Na) and potassium (K).

Hematology

Aliquots of whole blood for hematology analysis were stored at room temperature (up to 24 hr) and at 4 ± 1 °C (for 48, 72 and 96 hr) following blood withdrawal. Analysis was performed by a Coulter hematology auto-analyzer (Beckman Coulter, Galway, Ireland). Quality assurance controls were run on the analyzer throughout the study. The hematological parameters analyzed were total leucocyte count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT).

Statistical analysis

The results are presented in mean \pm standard deviation (SD) from data of 5 animals for both clinical chemistry and hematology. For statistical significance, results were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test to compare between zero hour and different experimental time points using GraphPad Prism software V.5 (San Diego, CA, USA).

Results

Table 1 presents the findings of clinical chemistry parameters obtained from samples stored at room temperature (23 ± 2 °C) for 6 hr and other samples at -20 ± 2 °C for 24 hr, 1 week, 2 weeks and 4 weeks respectively. The clinical chemistry parameters AST, ALP, ALT, CHOL, TRIG, CREAT, UREA, BUN, GLU, TP, GGT, T.BIL, PHOS, Na, K and Cl, did not show statistically significant changes in plasma stored at -20 °C for up to 4 weeks. However, ALT activity was decreased by 20.03% by week 4 in plasma stored at -20 °C. In contrast, LDH was increased by 15.15 %, 16.95 %, 49.39 % and 45.61 % after storage for 24 hr, 1 week, 2 weeks and 4 weeks respectively. GGT activity in plasma samples showed a variable trend at different storage time points, while a statistically significant ($P < 0.05$ or $P < 0.001$) decrease was noted in ALB at 1 and 2 weeks compared with initial time point (0 hr). A statistical significant decrease in calcium was observed at almost all the time points of storage (between 0 hr to week 4). The electrolyte values for Na, K and Cl were found to be consistent in all the samples (0 hr to 4 weeks).

Table 1. Clinical chemistry results of various parameters at different plasma storage times

Analyte	^a 0 hr	^a 6 hr	^b 24 hr	^b 1 week	^b 2 week	^b 4 week
ALB (g/dl)	4.14 \pm 0.11	4.06 \pm 0.11	4.06 \pm 0.09	3.92 \pm 0.08 **	3.84 \pm 0.09*	3.98 \pm 0.11
ALT (IU/L)	48.2 \pm 5.31	46.14 \pm 5.10	46.4 \pm 5.25	44.9 \pm 4.86	43.4 \pm 4.89	38.6 \pm 4.92
AST (IU/L)	75.8 \pm 7.46	72.2 \pm 5.98	75.1 \pm 7.45	78.4 \pm 8.05	80.8 \pm 9.29	78.4 \pm 8.07
ALP (IU/L)	268 \pm 108	259 \pm 103	264 \pm 104	268 \pm 105	261 \pm 104	253 \pm 101
CHOL (mg/dl)	59.3 \pm 8.54	58.20 \pm 8.76	59.2 \pm 9.33	57.5 \pm 9.18	58.5 \pm 8.92	60.8 \pm 8.66
TRIG (mg/dl)	92.8 \pm 22.4	90.8 \pm 21.7	92.0 \pm 20.52	93.6 \pm 20.3	94.8 \pm 20.5	95.3 \pm 19.5
CREAT (mg/dl)	0.66 \pm 0.03	0.64 \pm 0.06	0.64 \pm 0.02	0.62 \pm 0.03	0.61 \pm 0.03	0.62 \pm 0.02
UREA (mg/dl)	27.6 \pm 2.91	27.2 \pm 2.84	27.5 \pm 1.72	27.4 \pm 2.51	27.4 \pm 2.79	27.5 \pm 2.63
BUN (mg/dl)	12.9 \pm 1.36	12.7 \pm 1.32	12.8 \pm 0.80	12.8 \pm 1.17	12.8 \pm 1.30	12.9 \pm 1.23
LDH (IU/L)	334 \pm 86.0	337 \pm 75.2	384 \pm 95.7	390 \pm 77.6	499 \pm 146*	486 \pm 63.3
GLU (mg/dl)	136 \pm 10.1	133 \pm 9.98	135 \pm 9.44	132 \pm 10.6	131 \pm 9.09	134 \pm 9.48
TP (g/dl)	6.85 \pm 0.26	6.71 \pm 0.26	6.73 \pm 0.27	6.55 \pm 0.16	6.46 \pm 0.23	6.80 \pm 0.25
GGT (IU/L)	7.96 \pm 1.62	9.42 \pm 0.99	6.18 \pm 1.88	8.40 \pm 2.17	6.72 \pm 1.91	8.04 \pm 2.40
T.BIL (mg/dl)	0.12 \pm 0.02	0.15 \pm 0.05	0.11 \pm 0.03	0.14 \pm 0.04	0.13 \pm 0.02	0.12 \pm 0.03
CAL (mg/dl)	11.5 \pm 0.16	11.1 \pm 0.14*	11.8 \pm 0.17	10.9 \pm 0.25**	10.6 \pm 0.21**	11.0 \pm 0.15*
PHOS (mg/dl)	5.80 \pm 0.28	5.74 \pm 0.25	5.78 \pm 0.23	5.51 \pm 0.33	5.61 \pm 0.31	5.76 \pm 0.29
Na (mmol/l)	136.56 \pm 1.67	136 \pm 1.46	137 \pm 0.63	137 \pm 0.40	137 \pm 1.25	136 \pm 1.02
K (mmol/l)	3.99 \pm 0.34	3.97 \pm 0.33	4.01 \pm 0.35	3.99 \pm 0.34	4.00 \pm 0.34	4.02 \pm 0.36
Cl (mmol/l)	98.8 \pm 1.22	98.7 \pm 1.26	98.5 \pm 1.89	98.6 \pm 1.04	98.2 \pm 1.18	98.8 \pm 1.11

^a Time points of samples kept at 23 ± 2 °C

^b Time points of samples kept at -20 ± 2 °C

* $P < 0.05$, ** $P < 0.001$

Table 2. Haematology results of various parameters at different blood storage times

Analyte	^a 0 hr	^a 2 hr	^a 4 hr	^a 6 hr	^a 24 hr	^b 48 hr	^b 72 hr	^b 96 hr
WBC (10 ³ /μL)	12.1 ±1.14	12.0 ±1.52	12.0 ±1.42	11.6 ±1.04	11.6 ±1.30	11.9 ±0.94	13.1 ±1.80	13.0 ±1.26
RBC (10 ⁶ /μL)	8.48 ±0.29	8.45 ±0.21	8.53 ±0.28	8.50 ±0.37	8.54 ±0.24	8.17 ±0.37	8.19 ±0.23	8.51 ±0.19
HGB (g/dL)	14.8 ±0.67	14.9 ±0.42	14.9 ±0.59	14.8 ±0.67	15.0 ±0.40	15.0 ±0.51	15.2 ±0.42	15.1 ±0.33
HCT (%)	43.0 ±1.58	42.5 ±0.74	43.9 ±1.50	43.9 ±1.84	44.0 ±1.48	42.1 ±1.91	42.7 ±1.02	44.0 ±1.02
MCV (fL)	50.6 ±0.58	50.3 ±0.53	51.5 ±0.68	51.6 ±0.73	51.6 ±1.01	51.5 ±0.79	52.1 ±0.54*	51.8 ±0.55
MCH (pg)	17.5 ±0.33	17.7 ±0.11	17.5 ±0.22	17.4 ±0.15	17.5 ±0.19	18.3 ±0.54*	18.6 ±0.54**	17.7 ±0.29
MCHC (g/dL)	34.5 ±0.70	35.2 ±0.43	34.0 ±0.24	33.8 ±0.35	33.9 ±0.53	35.6 ±1.12	35.8 ±1.10	34.3 ±0.76
PLT (Thsn/μL)	964 ± 271	983 ±224	1078 ±158	1103 ±176	1078 ±126	1055 ±123	1010 ±104	1083 ±140

^a Time points of samples kept at 23 ±2 °C

^b Time points of samples kept at 4 ±1 °C

* P < 0.05, ** P < 0.001

Table 2 summarizes the results of hematology of various samples stored for analysis for 0, 2, 4, 6 and 24 hr (at room temperature, 23 ± 2 °C) or 48, 72 and 96 hr (at 4 ± 1°C). Complete blood count (CBC) analysis for various parameters in samples kept for the stated time intervals at 23 ± 2 °C to 4 °C revealed no statistically significant changes. However, a statistically significant (P<0.05) increase was observed for MCV that was 2.88 % at 72 hr, while values of MCH showed a statistically significant increase of 4.92 % at 48 hr and 6.52 % at 72 hr, in samples stored at 4 ± 1°C.

Discussion

Stability refers to the capability of a sample material to retain the initial property of a measured constituent for a period of time within specified limits when the sample is stored under defined conditions (ISO, 1992). The transport and storage condition of samples are crucial for testing and may affect the quality of the results. Storage of frozen samples has been recognized as an important factor in clinical pathology (Cray *et al.*, 2009). The present study was aimed to understand the influence of time and storage temperature on routinely analyzed biochemical and haematological parameters after preservation, which may have implications for the outcome of a laboratory investigation. The results for clinical chemistry revealed that the parameters AST, ALP, ALT, CHOL, TRIG, CREAT, UREA, BUN, GLU, TP, GGT, T.BIL, PHOS, Na, K and Cl, did not show statistically significant changes in plasma samples stored at 23 °C for 6 hr and at -20 °C for up to 4 weeks. Several analytes such as Na, protein, albumin, bilirubin, ALP, ALT, AST, GGT, creatinine kinase, lipase, CHOL, TGL etc. have been found to be stable under all storage con-

ditions (15-35 °C up to 24 hr) even before centrifugation (Tanner *et al.*, 2008). Certain analytes, in particular AST, have shown to exhibit higher activity in blood than in plasma (Sonntag, 1986).

In the present study, the increase in LDH activity in plasma of 15.15 % to 45.61 % from 24 hr to week 4 at -20 °C can be attributed to changes that occurred during storage, which is in agreement with LDH activity in human plasma which profoundly increased from 16 hr of storage onwards (Boyanton & Blick, 2002), and a similar trend was reported with delayed analysis of plasma and blood samples (Rendle *et al.*, 2009; Cray *et al.*, 2009; Peng *et al.*, 2010). A concomitant study also indicates that total LDH activity increased markedly after seven days of blood storage (Lindner & Hatzipanagiotou, 1993). In contrast, ALT activity was found to be decreased by 20.03 % in a sample stored for 4 weeks at -20 °C which is in agreement with a previous study that plasma ALT was stable up to 40 hr but then lost 20% activity at 48 and 56 hr (Boyanton & Blick, 2002). In our study, GGT activity in plasma samples showed a variable trend at different storage time points but were not statistically significant which can be compared to a study on human serum where GGT was found to be highly stable at 4 °C for 2 weeks and up to 4 months at -20 °C (Divya & Jayavardhan, 2010). However, statistically significant readings were noted with calcium at almost all the time points of storage (6 hr, or 1, 2 and 4 weeks) with lack of biological significance, and a statistically significant decrease was recorded in values of ALB after storage for 1 or 2 weeks. Previously, in a comparative biochemical analysis conducted in the ostrich (*Struthio camelus*), significantly lower levels of calcium were reported in citrated plasma than in serum (Mohri *et al.*,

2008). In line with this, significant changes after 24 hr have been recorded for albumin (+7 %, plasma and serum), calcium (+6 %, plasma) and total protein (+5 %, plasma) (Boyanton & Blick, 2002). In our haematology investigation, a 2.88 % increase was observed for MCV at 72 hr and 4.92 % for MCH at 48 hr and 6.52 % at 72 hr, respectively in samples stored at 4 °C. Comparable observations of a significant increase in MCV have been reported in stored (4 °C) blood samples of monkeys and rabbits at 72 hr, and in rats and mice at 24 hr (Ameri *et al.*, 2011). In addition, Hedberg & Lehto (2009) have demonstrated an increase of 10.6 % for MCV, 11.1 % for HCT and 11.1 % for MPV (mean platelet volume) values over time, and produced mean percentage changes over 10 % within 72 hr, suggesting changes in CBC parameters, which may be acceptable within ± 10 % of the original values, if not clinically significant. Samples of blood, serum, or plasma refrigerated (4 °C) for storage for up to a week, or frozen (≤ -20 °C) for prolonged preservation (Subramanian, 1995), even as long as 90 days, did not differ significantly for analytes (Cray *et al.*, 2009).

Conclusion

It is concluded that variations in the levels of analytes in stored blood and plasma from Wistar rats can be recorded and progressive changes with increase in duration of storage are likely to manifest as seen particularly with LDH. Nevertheless it was observed that preservation temperatures of -20 °C for plasma kept for up to 4 weeks and 4 °C for up to 96 hr for whole blood did not remarkably affect the stability of analytes except for LDH and may be considered for laboratory investigations if warranted.

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