BIOCHEMICAL VALUES STABILITY IN SERUM AND PLASMA OF RENAL DISEASED PATIENTS

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ABSTRACT

The stability of five parameters in serum and plasma after prolonged storage was studied by determining the effects of storage temperature and time on the laboratory results of samples obtained from renal diseased patients. Serum and plasma were separated from clot and red cells and analysed in duplicates immediately to obtain the baseline fresh value (zero hour value). The remaining serum and plasma were stored at $4^0C \pm 2^0C$ and $32^0C \pm 2^0C$ for 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 72, 96 and 120 hours, and then assayed in duplicates. Statistically significant changes from the zero hour mean value were determined using ANOVA. Instability ($P<0.05$) was observed at 32^0C for sodium and potassium ions after 96 hours, chloride after 72 hours, creatinine after 42hours and urea after 24 hours. All parameters in serum and plasma stored at 4^0C (refrigerated) were stable within 5 days. Proper storage temperatures and time must therefore be considered for these analytes if measurement does not to take place immediately after specimen collection.

Keywords: Analytes, Renal diseases, Blood Collection, Stability, Storage temperature, Storage time

INTRODUCTION

Laboratory tests are used by clinicians for diagnosis, monitoring, prognosis and treatment of patients suffering from different diseases (Mayne, 1994). A general problem facing clinical laboratories is the integrity of specimens used for biochemical assays. A number of factors such as preanalytical, analytical and normal biological variations affect the accuracy of laboratory test results (Stuart and Narayanan, 1996; Young and Bermes, 1999). Since analytical factors are being minimized by the development of new technologies, techniques and test kits, the relative contribution of preanalytical variation becomes a more dominant element in overall test variability. Preanalytical factors such as specimen collection, handling, storage and time of biochemical analysis all impact on test results leading to wrong judgment / diagnosis by the clinician (Stuart and Narayanan, 1996).

To detect real pathological changes in the patients, preanalytical variations must be reduced to acceptable levels at which they cause little or no impact on clinical interpretation of laboratory results (Heil *et al.,* 1995). Epidemiological studies can often be greatly enhanced by the inclusion of biochemical tests in serum and plasma specimens collected from the population being studied (Youngman *et al.,* 1993). For clinically useful and reliable test results, the interval between specimen collection, separation and subsequent analysis should be long enough to allow complete clot formation (for serum) but be shorter than the time in which a significant change is induced by

serum – clot and / or plasma – red cells contact (NCCLS, 1995).

Standard guidelines for blood sample handling state that serum and plasma should be physically separated from clot and red cells as soon as possible (for plasma) and immediately after clot formation is complete (for serum), unless conclusive evidence indicates that longer contact time do not contribute to result inaccuracy (Young and Bermes, 1999). NCCLS (1995) recommended a maximum clotting time of 20 - 30 minutes for serum. Whilst this is necessary for particular analytes, it might be assumed that many blood parameters deteriorate within a matter of hours in separated and unseparated samples kept at ambient temperature (Hankinson *et al.,* 1989).

Each individual analyte has a different tolerance to delay in sample processing. Since the laboratory receives the specimens mainly in the form of whole blood and then separates the serum and plasma from clot and red cells, the time interval between collection, separation and subsequent analysis must be controlled for reliable results to be obtained. It has been hypothesized that serum is more likely than plasma to be contaminated by haemolysis (Heard and Whitter, 1997). While heparinised plasma can be separated immediately after blood collection, serum must be harvested after a clot has formed (Thorensen *et al.,* 1992). However, an anticoagulant that binds an analyte may lower its measured value in plasma (Burkhard and Meyer, 1995).

Many investigators have studied related changes in some analytes during specimen storage (Saeed *et al*., 1995; Clark *et al.,* 2003; Marjani, 2008). The results are, however, controversial due to differences in ambient temperature conditions in different localities. No study known to the authors have been conducted in this area on these analytes in renal diseases patients. The primary objective of this study is therefore to determine whether prolonged storage similar to that encountered in field work and in the laboratory due to power outage affects the stability of sodium, potassium, chloride, urea and creatinine. A second objective is to determine whether there is a statistically significant difference between serum and plasma for biochemical assays of renal diseased patients.

MATERIALS AND METHODS

Approximately 10 ml of blood was collected by venepuncture between 8.00 and 9.00 hours from 40 fasting patients diagnosed with various forms of renal diseases at the Ebonyi State University Teaching Hospital, Abakaliki, Nigeria after their oral consent was obtained. After collection, 5 ml of the collected blood was dispensed into heparinised tube for plasma preparation. The other 5 ml was dispensed into a plain dry bottle for serum preparation. The heparinised bottle containing the blood was gently rocked to mix the heparin with the blood to avoid coagulation. The blood samples were then centrifuged at 3000xg for 10 minutes immediately (for plasma) and after 10 minutes (for serum). The plasma and serum were then collected into well labelled dry and clean pilot bottles.

The serum and plasma samples from each patient were analysed in duplicate immediately after separation from clot / red cells to obtain the baseline fresh values (zero hour value) against which other concentrations of future time-points were compared. The remaining serum and plasma were then divided into two equal parts. While one part was refrigerated at $4^0C \pm 2^0C$, the other part was kept on the laboratory bench at $32^0C \pm 2^0C$. Biochemical assays of the samples were carried out in duplicate at 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 72, 96 and 120 hour intervals.

Urea and creatinine levels in the serum and plasma samples were determined by the diacetyl monoxime method (Narsh *et al*., 1965) and Jaffe reaction (Bousnes and Taussky, 1975) respectively. Sodium and potassium ions concentrations were measured by the flame emission spectrophotometry (Ramsay *et al.,* 1953) while chloride levels were assayed by mercurimetric titration (Schales and Schales, 1944).

Mean values of these parameters were analysed using the SPSS package for windows version 18.0. Differences between the means were determined using analysis of variance

Table 1: Changes in the mean concentration of sodium ions (mmol/L) in specimens stored at 4^0C and 32^0C

Time	Serum		Plasma	
(hours)	4°C	32°C	4°C	32°C
0	150.0 ± 1.05	150.0 ± 1.11	150.0 ± 1.07	150.0 ± 1.00
4	150.0 ± 0.90	150.0 ± 0.79	150.0 ± 1.16	150.0 ± 1.05
8	150.0 ± 0.18	150.0 ± 1.66	150.0 ± 0.92	150.0 ± 1.02
12	150.0 ± 1.08	149.0 ± 1.52	150.0 ± 0.51	149.0 ± 1.40
16	150.0 ± 0.73	149.0 ± 2.81	150.0 ± 1.72	149.0 ± 1.13
20	150.0 ± 1.56	148.0 ± 1.93	150.0 ± 1.20	148.0 ± 2.20
24	150.0 ± 1.14	148.0 ± 1.44	150.0 ± 1.58	147.0 ± 1.54
30	150.0 ± 1.22	147.0 ± 2.15	150.0 ± 0.98	146.0 ± 1.78
36	150.0 ± 1.61	146.0 ± 0.98	150.0 ± 1.24	145.0 ± 0.91
42	150.0 ± 0.54	145.0 ± 1.67	150.0 ± 0.91	144.0 ± 2.41
48	150.0 ± 0.65	144.0 ± 1.84	150.0 ± 0.74	143.0 ± 1.64
72	149.0 ± 0.78	141.0 ± 1.57	149.0 ± 1.22	138.0 ± 1.66
96	149.0 ± 0.81	137.0 ± 0.98	149.0 ± 1.41	133.0 ± 1.11
120	148.0 ± 1.72	132.0 ± 1.69*	148.0 ± 0.86	130.0 ± 1.70*

Asterisks (*) superscript on a mean indicates that it is significantly different from the hour zero value (P<0.05)

Table 2: Changes in the mean concentration of potassium ions (mmol/L) in specimens stored at 4^0C and 32^0C

Time	Serum		Plasma	
(hours)	4°C	32°C	4°C	32°C
0	5.50 ± 0.57	5.50 ± 0.98	5.50 ± 0.15	5.50 ± 1.08
4	5.50 ± 0.41	5.50 ± 0.80	5.50 ± 0.44	5.50 ± 1.67
8	5.50 ± 1.93	5.50 ± 1.31	5.50 ± 1.76	5.50 ± 0.51
12	5.50 ± 0.67	5.50 ± 1.54	5.50 ± 1.41	5.50 ± 0.32
16	5.50 ± 1.72	5.50 ± 1.23	5.50 ± 1.89	5.50 ± 1.40
20	5.50 ± 1.30	5.40 ± 1.52	5.50 ± 0.86	5.40 ± 1.72
24	5.50 ± 1.54	5.40 ± 2.42	5.50 ± 0.32	5.40 ± 1.12
30	5.50 ± 1.76	5.40 ± 1.16	5.50 ± 0.78	5.40 ± 1.84
36	5.50 ± 1.88	5.30 ± 1.55	5.50 ± 1.31	5.30 ± 1.65
42	5.50 ± 1.01	5.30 ± 2.18	5.50 ± 1.95	5.30 ± 2.42
48	5.50 ± 1.17	5.30 ± 1.96	5.50 ± 1.48	5.30 ± 1.90
72	5.40 ± 1.51	5.10 ± 0.87	5.40 ± 1.56	5.00 ± 1.31
96	5.40 ± 0.93	4.90 ± 1.52	5.40 ± 1.21	4.80 ± 1.96
120	5.30 ± 0.64	4.70 ± 1.98*	5.30 ± 1.98	4.50 ± 1.83*

Asterisks (*) superscript on a mean indicates that it is significantly different from the hour zero value (P<0.05)

Table 3: Changes in the mean concentration of chloride ions (mmol/L) in specimens stored at 4^0C and 32^0C

Time	Serum		Plasma	
(hrs)	4°C	32°C	4°C	32°C
0	113.0 ± 1.41	113.0 ± 1.50	113.0 ± 1.94	113.0 ± 1.79
4	113.0 ± 1.51	113.0 ± 3.10	113.0 ± 1.85	133.0 ± 1.91
8	113.0 ± 0.72	113.0 ± 1.91	113.0 ± 1.16	112.0 ± 2.08
12	113.0 ± 1.02	112.0 ± 2.16	113.0 ± 1.57	112.0 ± 1.46
16	113.0 ± 1.61	112.0 ± 1.68	113.0 ± 0.62	111.0 ± 1.74
20	113.0 ± 1.53	111.0 ± 1.60	113.0 ± 1.30	110.0 ± 1.55
24	113.0 ± 0.19	110.0 ± 2.86	113.0 ± 1.71	109.0 ± 2.56
30	113.0 ± 1.05	109.0 ± 1.11	113.0 ± 0.42	108.0 ± 0.89
36	112.0 ± 1.33	108.0 ± 1.77	112.0 ± 1.60	107.0 ± 1.67

42	112.0 ± 0.96	107.0 ± 1.76	112.0 ± 0.83	106.0 ± 4.18
48	112.0 ± 1.07	106.0 ± 2.89	112.0 ± 0.39	105.0 ± 1.97
72	111.0 ± 1.54	101.0 ± 1.56	111.0 ± 0.11	99.0 ± 1.66
96	111.0 ± 1.06	95.0 ± 0.98*	111.0 ± 1.24	92.0 ± 3.46*
120	110.0 ±0.51	88.0 ± 1.55*	110.0 ± 0.45	84.0 ± 1.82*

Asterisks (*) superscript on a mean indicates that it is significantly different from the hour zero value (P<0.05)

Table 4: Changes in the mean concentration of urea (mmol/L) in specimens stored at 4^0C and 32^0C

Time	Serum		Plasma	
(hrs)	4^oC	32^oC	4^oC	32^oC
0	9.20 ± 1.41	9.20 ± 1.98	9.20 ± 1.78	9.20 ± 1.13
4	9.20 ± 1.14	9.10 ± 2.01	9.20 ± 0.65	9.10 ± 1.95
8	9.20 ± 0.32	9.00 ± 1.86	9.20 ± 1.09	9.00 ± 2.23
12	9.20 ± 1.06	8.90 ± 2.42	9.20 ± 0.88	8.90 ± 1.67
16	9.20 ± 0.89	8.80 ± 1.33	9.20 ± 1.06	8.80 ± 3.11
20	9.20 ± 0.63	8.70± 1.22	9.20 ± 0.54	8.70 ± 1.09
24	9.20 ± 1.54	8.60 ± 2.07	9.20 ± 1.01	8.60 ± 1.70
30	9.20 ± 1.93	8.30 ± 0.96*	9.20 ± 2.00	8.20 ± 4.07*
36	9.20 ± 0.87	8.00± 1.23*	9.20 ± 1.84	7.90 ± 1.10*
42	9.10 ± 1.10	7.70 ± 2.11*	9.10 ± 0.97	7.50 ± 0.89*
48	9.10 ± 2.14	7.40 ± 1.01*	9.10 ± 1.11	7.10 ± 1.88*
72	9.10 ± 0.36	6.10 ± 1.56*	9.10 ± 0.91	5.80 ± 1.50*
96	9.00 ± 1.07	4.00 ± 1.87*	9.0 ± 1.46	3.70 ± 2.66*
120	8.90 ± 1.15	2.80 ± 1.84*	8.90 ± 1.23	2.20 ± 1.42*

Asterisks (*) superscript on a mean indicates that it is significantly different from the hour zero value (P<0.05)

Table 5: Changes in the mean concentration of creatinine (µmol/L) in specimens stored at 4^0C and 32^0C

Time	Serum		Plasma	
(hrs)	4^oC	32^oC	4^oC	32^oC
0	133.0 ± 1.51	133.0 ± 1.66	133.0 ± 1.11	133.0 ± 1.54
4	133.0 ± 1.44	132.7 ± 1.45	133.0 ± 1.32	132.7 ± 0.98
8	133.0 ± 0.56	132.4 ± 1.51	133.0 ± 0.76	132.3 ± 1.46
12	133.0 ± 0.74	132.0 ± 1.32	133.0 ± 1.43	131.9 ± 1.08
16	133.0 ± 0.86	131.5 ± 2.01	133.0 ± 1.80	131.4 ± 3.06
20	133.0 ± 0.63	131.0 ± 1.54	133.0 ± 1.20	130.8 ± 1.25
24	133.0 ± 0.46	130.5 ± 1.30	133.0 ± 1.16	130.2 ± 2.02
30	133.0 ± 1.14	130.0 ± 0.99	133.0 ± 0.66	129.5 ± 1.66
36	133.0 ± 1.42	129.0 ± 1.08	133.0 ± 1.11	128.8 ± 0.98
42	132.0 ± 0.88	128.0 ± 3.52	132.0 ± 1.67	127.6 ± 1.75
48	132.0 ± 0.67	127.0 ± 1.73*	132.0 ± 0.85	126.4 ± 1.34*
72	132.0 ± 1.73	117.0 ± 1.64*	132.0 ± 0.81	114.0 ± 1.09*
96	131.0 ± 1.11	97.0 ± 2.22*	131.0 ± 1.17	93.0 ± 1.76*
120	130.0 ± 1.30	65.0 ± 1.01*	130.0 ± 1.81	58.0 ± 2.08*

Asterisks (*) superscript on a mean indicates that it is significantly different from the hour zero value (P<0.05)

(ANOVA). Differences with P<0.05 were accepted as significant. The results obtained from samples stored at 4 ± 2^0C were also compared with that obtained from samples stored at 32 ± 2^0C at each time interval using paired sample student t-test. Also, the serum and plasma values per unit time were compared with each other using the student t-test.

RESULTS AND DISCUSSION

There is a lack of consensus regarding the most appropriate specimen type for analysis of many biochemical parameters. Information on the stability of serum and plasma analytes during storage is often incomplete and sometimes contradictory. This study was carried out to determine the effects of storage on the concentrations of sodium, potassium, chloride, urea and creatinine with the view to compare the stability of these analytes in serum and plasma specimens after prolonged storage.

The mean concentrations of sodium and potassium ions in specimens stored at $32^0C \pm 2^0C$ decreased significantly ($P<0.05$) from the fresh value after 96 hours of storage (Tables 1 and 2). Again, this change was more pronounced in plasma samples. This study has also shown that the mean chloride ion levels (Table 3) decreased significantly ($P<0.05$) from the zero hour value after 72 hours of specimen storage at ambient temperature, with instability being more marked in plasma.

The effect of storage on the levels of these analytes in serum and plasma is dependent on incubation temperature. Urea concentration was stable for up to 24 hours of storage at $32^0C \pm 2^0C$, after which statistically significant ($P<0.05$) decrease was observed (Table 4), with the degree of change being more pronounced in plasma. Statistically significant decrease ($P<0.05$) was observed for creatinine levels after 42 hours of storage at $32^0C \pm 2^0C$ (Table 5), with the degree of change being more pronounced in plasma. These analytes were, however, stable throughout the storage period when specimens were refrigerated, implying that their stability during storage depends on storage temperature.

This study has therefore demonstrated that urea and creatinine levels can be measured reliably in serum / plasma specimens kept at room temperature if assayed within 24 and 42 hours of sample collection respectively. Furthermore, reliable quantification of sodium, potassium and chloride ions in serum and plasma requires that analysis be carried out within 96, 96 and 72 hours respectively if specimen is stored at $32^0C \pm 2^0C$. Since these biochemical parameters exhibited significantly greater stability when specimens were kept at $4^0C \pm 2^0C$, reliable results for diagnosis and prognosis can still be obtained up to 5 days when refrigerated.

Our findings for the stability of these analytes in serum and plasma are consistent with those reported by earlier investigators in serum and plasma stored after immediate separation from clot / red cells (Donnelly *et al*., 1995; Heins *et al*., 1995; Saeed *et al*., 1995; Boyanton and Blick, 2002). Contrary to this finding, Zhang *et al.* (1998) observed that sodium ion concentrations increased, though non-significantly after 24 hours of serum - clot contact at $32^0C \pm 2^0C$. This was attributed to haemoconcentration due to the movement of water into cells after 24 hours of serum-clot contact. Zhang *et al.* (1998) also reported that potassium ions fluctuated with an initial decrease followed by a large increase after 6 hours at $32^0C \pm 2^0C$. Similarly, Day *et al.* (2001) observed rise in potassium levels but attributed this to the net effect of glycolysis which moves potassium ions into the cells and passive diffusion, which allows potassium ions to diffuse out of the cells after longer serum – clot contact.

Studies by Laessig *et al.* (1976) and Zhang *et al.* (1998) indicated that serum was suitable for chloride ion determination only within 6 hours of serum – clot contact when stored at $32^0C \pm 2^0C$. We, however, observed chloride ions to be stable for 72 hours at $32^0C \pm 2^0C$. This discrepancy may be explained by differences in study design since Laessig *et al.* (1976) and Zhang *et al.* (1998) performed biochemical assays on sera after prolonged contact with clot. The sudden fall in chloride ion levels observed by these researchers is likely due to the chloride – bicarbonate shift with subsequent buffering of hydrogen ions (from lactic acid) by bicarbonate with the production of carbon dioxide.

The loss of sodium, potassium and chloride ion concentrations observed in the present study when specimens were stored at ambient condition may be attributable to bacterial contamination. Since the specimen containers are not sterile, bacteria present in

them may have used up these ions for their normal metabolic activities (Schwartz, 1993). As such, proper storage temperature and time if assay is not to take place immediately after specimen collection and separation was recommended (Donnelly *et al*., 1995).

Investigating the stability of urea and creatinine in human serum and plasma after prolonged contact with clot and red cells, Boyanton and Blick (2002) noted non-significant increase in urea level after 24 hours of contact at 25^0C. These researches also reported that creatinine levels increased by 110% in plasma and 60% in serum after 24 hours of contact with cells at 25^0C. Similarly, Clark *et al.* (2003) observed creatinine levels to rise by more than 20% in plasma after 3 days of delayed separation from whole blood at 21^0C.

By contrast, in the present study, urea and creatinine levels decreased in serum and plasma during storage at ambient condition. Bacterial contamination and subsequent deterioration are possible reasons for the loss of these analytes at 32^0C ± 2^0C (Schwartz, 1993). Discrepancies between the findings of Boyanton and Blick (2002), and Clark *et al.* (2003) with the present study might be explained by differences in study design. These investigators performed biochemical assays on serum / plasma samples after prolonged contact with clot / red cells, rather than on serum / plasma samples stored for prolonged time after immediate separation from clot / red cells, as in the present study.

Furthermore, the differences in the stability of these analytes between the serum and plasma samples is worthy of note. Although a statistically significant difference was not noted in this study between serum and plasma specimens ($P>0.05$), these parameters were observed to be more stable in serum at 32^0C ± 2^0C. Similar differences in stability between constituents in serum and plasma has been reported (Juul, 1997). The investigator concluded that the higher instability of plasma constituents was due to the effect of the anticoagulant used. Boyanton and Blick (2002) also recommended the use of serum whenever prolonged contact of serum or plasma with cells is unavoidable due to the higher instability of plasma analytes. Boyanton and Blick (2002) went further to demonstrate that after immediate separation of plasma and serum from cells, however, there was no significant difference between the stability of analytes in plasma and serum provided they are stored under similar conditions.

Conclusion: The stability of a wide range of blood analytes in serum / plasma stored for several hours is much better than had perhaps been widely believed. It is hoped that these findings will help to determine which of the constituents of serum / plasma that may be assayed in specimens stored for prolonged time when such prolonged storage is unavoidable. However, recording the length of time from sample collection to biochemical analysis for each sample might allow appropriate adjustment to be made for the loss in the concentration of these parameters on storage. Finally, the use of serum is recommended particularly when prolonged storage is unavoidable or occurs inadvertently.

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