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Sample management: stability of plasma and serum on different storage conditions

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ABSTRACT

Background and objective

The analytes stability on serum and plasma are critical for clinical laboratory, especially if there is a delay in their processing or if they need to be stored for future research. The objective of this research was to determine the stability of K_3EDTA-plasma and serum on different storage conditions.

Materials and methods

A total of thirty healthy adults were studied. The serum/plasma samples were centrifuged at 2000g for 10 minutes. Immediately after centrifugation, the serum/plasma analytes were assayed in primary tubes using a Cobas c501 analyzer (T0); the residual serum/plasma was stored at either 2-8°C or -20°C for 15 (T15) and 30 days (T30).

Mean concentrations changes in respect of initial concentrations (T0) and the reference change values were calculated. For assessing statistical difference between samples, the Wilcoxon ranked-pairs test was applied.

Results

We evidenced instability for total bilirubin, uric acid, creatinine and glucose at T15 and T30 and stored at -20°C ($p<0.05$). However, potential clinical impact significance were observed only for total bilirrubin T30 at -20°C, and creatinine T30 at 2-8°C.

Conclusions

Our results had shown that storage samples at -20°C is a better way to preserve glucose, creatinine, and uric acid. Therefore, laboratories should freeze their samples as soon as possible to guarantee proper stability when there is need to repeat analysis, verify a result, or add a laboratory testing.

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INTRODUCTION

Since the 1960s, the study of analyte stability has been considered a critical aspect for laboratory medicine, since variations due to lack or reduced stability reflect on the results of laboratory tests employed for taking clinical or therapeutic decisions for patients (1,2).

The stability of an analyte in clinical biochemistry can be defined as "the space of time in which it maintains its value within established limits, by storing the sample in which the analytes are analyzed under certain specific conditions" (3,4). Currently, between 60-80% of medical decisions are based on the results obtained by the laboratory (5).

Moreover, it is well known that the pre-analytical phase is most susceptible to errors possibly leading to the unexpected variations of laboratory testing in biological samples; indeed, it is considered responsible for approximately 70% of the total errors in clinical laboratories (6).

Laboratories should use to quantify the analytes fresh serum or plasma in order to avoid degradation. However, the use or re-use of primary samples previously obtained from patients can be needed in the following situations:

i) due to delay in the analysis procedure;

ii) to confirm or to check a previously obtained value;

iii) to add new quantifications of missing analytes.

Therefore, this may lead to false concentration measurements (7), in case of uncontrolled storage conditions. On other occasions, the samples are transferred from more or less distant places, and under different storage conditions until the moment of the analysis; which several times do not correspond to quality standards (i.e., temperature control) (8,9). All of this causes the matrix analysis to undergo changes or degradation processes, which can be cumulative over time and possibly leading to alterations of original sample results (10).

Prolonged contact of plasma or serum with cells is a common cause of variability on test results (11). Plasma and serum should preferably be separated from cells as quickly as possible to avoid the ongoing metabolism of cellular constituents, as well as the active and passive movements of analytes between plasma or serum and cell compartments (12) This study was planned to verify the stability of K_3EDTA-plasma and serum on different storage conditions.

MATERIALS AND METHODS

Blood samples were obtained from 30 healthy adult laboratory workers (20 women and 10 men; average age was 33 [21-50] years), after signing informed consent. The inclusion criteria were not to suffer from infectious or chronic diseases, not to be on antibiotic treatment or any other medication for any acute illness. Moreover, 12 hour overnight fasting was required (13, 14). Blood samples where obtained in the morning

Table 1 Results of within-run precision by internal quality control

Tests	Method	IQC assigned values	CVa (%)	CVi (%)
Glucose	Enzymatic Colorimetric – Hexokinase	5.78 mmol/L	2.2	5.6
Creatinine	Kinetic Colorimetric Assay - Jaffé (2-compensated method)	90.5 µmol/L	4.6	5.9
Uric acid	Enzymatic colorimetric test - Uricase –Peroxidase	269 mmol/L	1.5	8.6
Total Bilirubin	Colorimetric assay – DPD (Diazonium salt)	17.8 µmol/L	4.0	21.8
Direct Bilirubin	Colorimetric assay – DPD (Diazo reaction)	17.1 µmol/L	3.2	36.8

by two phlebotomists with a puncture of the anterior bend of the elbow using the multiple extraction device (Vacuette®), Vacuette® 21G needle: 2 evacuated tubes with K3EDTA (4 mL), and 2 with clot activator and gel separator (8 mL), for plasma and serum collection respectively; samples were mixed by gently inversion (15). The tubes were placed vertically in a rack for 30 minutes at room temperature (24 °C) and then centrifuged at 2000 g for 10 minutes. All samples were not hemolyzed, icteric or lipemic.

In the primary serum and K_3EDTA-plasma tubes (T0, used for reference measurement), five clinical chemistry analytes – glucose, uric acid, creatinine, direct bilirubin and total bilirubin – were quantified in duplicate on Cobas® c501 autoanalyzer from Roche Diagnostics (Germany). The equipment was previously calibrated and controlled according to our own analytical quality assurance procedure established by the laboratory's management system in NetLab (Synlab Ecuador) Accredited Specialty Laboratory under ISO 15189 standards (Table 1) (16).

Serum and plasma samples were aliquoted in sterile plastic tubes with lids. Two aliquots of serum and two aliquots of plasma were stored at 2-8°C and two aliquots of serum and two aliquots of plasma were stored at -20°C. Therefore, four aliquots per patient and per matrix were stored for 15 days (T15) and 30 days (T30), this process prevents freeze-thawing of the samples in each analysis. Moreover, all aliquots were protected from light. Prior to the experimental stability measurements, the aliquots were placed at room temperature (24 °C) for 45 minutes to thaw and then the five biochemical parameters in serum and plasma were analyzed at the same time, with the same methodology and under the same conditions as the reference measurement (T0).

The mean and standard deviation were calculated for each analyte. The coefficient of variation (CVa%) of each analyte was the cumulative of the internal quality control data (last six months) (17,18), using third-party control materials-independent from calibrator materials-either (19). For assessing statistical difference between samples, the Wilcoxon ranked-pairs test was used. Difference % mean was calculated using the formula:

$$(T_x - T_0)/(T_0)\,x100\ \%$$

T_0: Initial median value

T_x: Median value of the measured values at T15 or T30

Finally, the reference change value (RCV) was applied for the analysis of the potential clinical impact.

The following formula was used for its calculation:

$$RCV = 2^{1/2} \times Z \times \left(CVa^2 + CVi^2\right)^{1/2}$$

It corresponds to the square root of 2, the statistic Z = 1.9 (obtained from the normal distribution table, with a 95% confidence) and the coefficient of intra-individual biological variation (CVi) of the analyte being assayed (20). The last factor is the laboratory's own coefficient of analytical variation (CVa%). When the mean percentage difference of an analyte is less than the RCV calculated, one infers that there are no errors in the last quantification; whereas if mean percentage difference is bigger than the RCV calculated, this mirror potential clinical impact; in our study it refers to instability of the analyte evaluated.

The difference between serum and K_3EDTA-plasma were evaluated by paired t-test on T0. The collected data were processed in a data matrix in Excel (Microsoft) and statistical analysis were performed with the program SPSS v.18 (SPSS Inc.).

RESULTS

We evidenced instability for total bilirubin, uric acid, creatinine and glucose at T15 and T30 and stored at -20°C ($p<0.05$). However, potential clinical impact significance were observed only for total bilirubin T30 at -20°C, and creatinine T30 at 2-8°C. Statistical analysis of serum versus K_3EDTA-plasma did not show significant difference ($p>0.05$); Table 2.

DISCUSSION

The results of this study showed that the concentrations of the analytes in serum and K3EDTA-plasma were equivalent. Our results regarding differences between serum and plasma were similar with other studies (21,22), also if they used heparinized plasma instead of K3EDTA-plasma. In the past, uncertainty regarding the stability of serum analytes was a major concern because serum has been the preferred specimen by most laboratories. However, the serum specimens have several inherent problems, such as the time necessary for clot formation: at least 30 minutes (12, 23, 24). Several studies prefer lithium heparin plasma for the analysis of routine clinical chemistry (11, 25), particularly for the analysis of certain analytes (i.e., phenytoin) (21).

The inclusion of K3EDTA-plasma in our study is due to the observed need to obtain data in this matrix. Inpatients with hypovolemic shock generally require biochemical and hematological tests, however is difficult to obtain the number of samples and volume necessary for these analytical tests (whole blood for hematology and serum for chemistry).

Therefore, in these clinical conditions, it could be enough to take a single tube of blood sample (i.e., K_3EDTA) for the required tests. Obviously, electrolytes cannot be measured in this sample matrix, since EDTA is an important chelate (26).

The World Health Organization further states that heparin plasma samples either are not recommended for all analytical methods that include glucose and others (27). Over time, several studies support the fact that serum is preferred as the matrix analysis by most clinical laboratories (28).

Boyanton & Blick evaluated the stability of 24 analytes in serum and plasma where several analytes remained stable in both matrices for a period of 56 hours; the greatest change was observed for direct bilirubin (12).

Table 2 Analyte concentration by sample type (serum or plasma EDTA), storage time (T0, T15 and T30) and temperature of conservation

Analyte	Serum						K_3EDTA Plasma					
	Stability	median [interquartile range]	mean % difference	p	RCV	Potential clinical impact	Stability	median [interquartile range]	mean % difference	p**	RCV	Potential clinical impact
Glucose 2-8°C *(mmol/L)*	T0	4.86 [4.32–5.23]	-	-	-	-	T0	5.02 [4.48–5.35]	-	-	-	-
	T15	5.22 [4.66–5.73]	7.41	<0.001	16.4	**No**	T15	5.19 [4.70–5.62]	3.38	<0.001	16.4	No
	T30	5.05 [4.36–5.33]	3.91	<0.001	-	No	T30	4.97 [4.33–5.40]	-0.99	0.202	-	No
Glucose -20°C *(mmol/L)*	T0	4.86 [4.32–5.23]	-	-	-	-	T0	5.02 [4.48–5.35]	-	-	-	-
	T15	4.96 [4.37–5.23]	2.06	0.005	16.4	No	T15	5.16 [4.69–5.44]	2.78	0.010	16.4	No
	T30	4.72 [4.14–5.11]	2.88	<0.001	-	No	T30	4.97 [4.44–5.26]	-0.99	0.037	-	No
p-Value serum vs. plasma EDTA*	0.304											

Creatinine 2-8°C *(µmol/L)*	T0	64.1 [53.0–79.8]	-	-	-	-	T0	63.7 [53.9–78.2]	-	-	-	-
	T15	75.1 [61.0–92.4]	17.2	<0.001	20.9	**No**	T15	71.6 [58.3–85.3]	12.4	<0.001	20.9	**No**
	T30	78.2 [63.4–92.2]	22.0	<0.001	-	Yes	T30	72.1 [59.2–87.7]	13.2	<0.001		**No**
Creatinine -20°C *(µmol/L)*	T0	64.1 [53.0–79.8]	-	-	-	-	T0	63.7 [53.9–78.2]	-	-	-	-
	T15	68.9 [56.6–84.2]	7.49	<0.001	20.9	No	T15	66.7 [54.6–83.1]	4.70	<0.001	20.9	No
	T30	70.7 [59.0–84.2]	10.3	<0.001	-	No	T30	71.2 [57.2–86.9]	11.8	<0.001	-	**No**
p-Value serum vs. plasma EDTA*						0.844						
Uric acid 2-8°C *(mmol/L)*	T0	0.24 [0.22–0.29]	-	-	-	-	T0	0.23 [0.20–0.26]	-	-	-	-
	T15	0.28 [0.24–0.32]	16.7	<0.001	24.2	**No**	T15	0.24 [0.22–0.30]	4.3	<0.001	24.2	No
	T30	0.28 [0.24–0.32]	16.7	<0.001	-	No	T30	0.24 [0.22–0.29]	4.3	<0.001	-	**No**
Uric acid -20°C *(mmol/L)*	T0	0.24 [0.22–0.29]	-	-	-	-	T0	0.23 [0.20–0.26]	-	-	-	-
	T15	0.25 [0.23–0.30]	4.16	<0.001	24.2	No	T15	0.24 [0.21–0.28]	4.3	<0.001	24.2	No
	T30	0.26 [0.23–0.30]	8.33	<0.001	-	No	T30	0.25 [0.21–0.29]	8.7	<0.001	-	No
p-Value serum vs. plasma EDTA*						0.212						

Total Bilirubin 2-8°C *(µmol/L)*	T0	7.27 [5.09–12.7]	-	-	-	-	T0	7.35 [4.92–12.1]	-	-	-	-
	T15	4.79 [3.08–9.45]	-34.1	<0.001	61.5	**No**	T15	3.42 [2.27–6.58]	-53.5	<0.001	61.5	No
	T30	2.57 [1.33–4.66]	-64.6	<0.001	-	No	T30	3.93 [1.88–6.84]	-46.5	<0.001	-	No
Total Bilirubin -20°C *(µmol/L)*	T0	7.27 [5.09–12.7]	-	-	-	-	T0	7.35 [4.92–12.1]	-	-	-	-
	T15	5.05 [3.38–11.4]	-30.5	<0.001	61.5	No	T15	6.24 [4.02–11.4]	-15.1	<0.001	61.5	No
	T30	1.45 [0.68–4.53]	-80.1	<0.001		Yes	T30	1.54 [0.86–3.51]	-79.0	<0.001		Yes
p-Value serum vs. plasma EDTA*						0.759						
Direct Bilirubin 2-8°C *(µmol/L)*	T0	2.99 [2.57–5.13]	-	-	-	-	T0	2.48 [2.01–3.81]	-	-	-	-
	T15	2.05 [1.45–3.63]	-31.4	<0.001	102.4	**No**	T15	1.37 [0.94–2.22]	-44.8	<0.001	102.4	No
	T30	0.86 [0.34–1.71]	-71.2	<0.001	-	No	T30	0.86[0.51–1.24]	-65.3	<0.001	-	**No**
Direct Bilirubin -20°C *(µmol/L)*	T0	2.99 [2.57–5.13]	-	-	-	-	T0	2.48 [2.01–3.81]	-	-	-	-
	T15	2.65 [2.05–4.32]	-11.4	0.057	102.4	No	T15	2.39 [1.84–3.76]	-3.62	0.057	102.4	No
	T30	1.80 [0.98–2.74]	-39.8	<0.001	-	No	T30	1.80 [1.37–2.82]	-27.4	<0.001	-	No
p-Value serum vs. plasma EDTA*						0.078						

** = Student T test; ** = Wilcoxon ranked-pairs test.*

Our results had shown concentrations decrease on total bilirubin and direct bilirubin. However, this change could be produced by photo degradation (29).

Similar data were found by Tambse et al., who reported 10% variation for bilirubin in samples stored for 18-20 days at 4-8°C; whereas glucose, uric acid and creatinine showed an increase concentration over time (30).

A further study by Heins et al. reports that, from 22 analytes studied in serum, bilirubin stored at room temperature for 7 days had the greatest variation in concentration (31). Despite this, they conclude that all concentrations of the analytes studied including uric acid, creatinine and bilirubin are stable for four days in serum samples that were separated from the cells and stored at 9°C (31); whereas our results were different (Table 2).

Probably the increased concentrations observed in our study is due to evaporation of the samples stored. Similar results were evidenced by Alcaraz et al., after stored serum samples at 4°C for 5 days (32).

Only creatinine presented a potential clinical impact in T30 at 2-8°C (Table 2). In contrast with previous study described by Kachhawa et al., who report no clinical differences in creatinine, and uric acid concentrations between the mean values of day 1 and T7, T15 and T30 stored at -20°C (33). Thus demonstrating that the optimal storage condition is obtained by freezing the biological samples, since it could decrease the activity of some proteolytic enzymes that can alter the structure of the analytes (34).

Monneret et al. had shown good samples stability for 3, 4, 5, and 6 hours at room temperature in total blood and plasma (lithium heparin) for creatinine, total bilirubin and uric acid testing (25).

The analysis of the potential clinical impact in plasma at T15 and T30 stored at -20°C showed no significant difference for glucose, uric acid and direct bilirubin; demonstrating that the variations in concentrations are equivalent to the concentrations found in serum.

These results are consistent with those reported by other researchers (2, 21, 35, 36). Therefore, its necessary for each laboratory to evaluate the specific conditions and to define the storage time for any analyte to guarantee the stability. This will properly lead physicians to perform diagnostic, follow up, or therapeutic management.

In conclusion, our results had shown that storage samples at -20°C is a better way to preserve glucose, creatinine, and uric acid. Therefore, laboratories should freeze their samples as soon as possible to guarantee properly stability when there is a need to repeat analysis, verify a result, or add a laboratory testing.

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