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TO DETERMINE THE EFFECT OF STORAGE TEMPERATURE ON THE STABILITY OF SERUM ANALYTES

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ABSTRACT

Background and Objective: In the clinical laboratories, stability of serum analytes is most common problem during sample storage. Samples are usually stored either at 4–8°C in a refrigerator for short durations or in a deep freezer at −20°C for longer periods. So, the temperature at which the samples are stored constitutes an important pre-analytical variable that may affect analysis results in the clinical biochemistry laboratory setting. The objective of the present research is to determine the stability of serum in different storage conditions. ***Materials and Methods:*** A total of fifty healthy volunteers were studied. Serum samples were separated in aliquots with a lid. 8 aliquots of serum were stored at 4^0C and -20^0C. Therefore, 8 aliquots per patient were stored for 1, 7, 15, and 30 days. Urea, Creatinine, Total Protein, Albumin, Triglyceride, Sodium, Potassium, Chlorine, and total, Direct, and Indirect Bilirubin were measured by using ongoing methods performed in the clinical biochemistry laboratory. ***Results:*** We evidenced the instability of Creatinine, Total Protein, Albumin, Sodium, Potassium, Chlorine, and Total, Direct, and Indirect Bilirubin at both the temperature (4^0C and -20^0C). However, potential clinical impact significance was observed only for urea at T7 stored at -20^0C. ***Conclusions:*** Our results showed that Samples should analyze in the laboratory within preferably 24 h of collection to ensure valid results. In addition, the turn-around time from sample drawing to reporting the analytical result would be shortened.

KEYWORDS: Stability, analytes, serum.

INTRODUCTION

The definition of stability of analytes especially for biochemistry can be "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."

Maintaining the stability of serum analytes during sample storage is most common issue in any clinical laboratory. Samples are usually stored either at 4–8°C of a refrigerator for short durations or in a deep freezer at −20°C for longer periods. So, the temperature at which the samples are stored constitutes an important pre-analytical variable that may affect analysis results in the clinical biochemistry laboratory setting.[1]

Laboratories of tertiary care hospitals received over a thousand samples a day. These laboratories are facing so many challenges like a breakdown of equipment or lack of reagents or lack of staff, which can prevent same-day processing of samples.

Previous studies have provided information regarding the stability of analytes in serum using several methods,[2,3] that have since become venerable. Studies reported short-term stability of common biochemical analytes in human serum specimens if the samples were stored in the refrigerator (at 4 °C) and/or at room temperature (RT),[4,5] Prolonged storage and long-term stability of biochemical analytes samples requires deep freezer at -20°C to -80°C temperature as compared to the storage temperature of 4°C or RT.[6,7,8] The temperature and length of storage are important factors that may affect the outcome of serum biochemical examination.

Data concerning the stability of serum analytes aboutthe time duration and temperatures of storage in human serum samples are scarce.[9]

Information available regarding the stability of commonly used clinical biochemical analytes in human serum including the effect of storage temperatures as low as −20°C on blood-separated serum is very low.

Therefore, this study was carried out to address the possible storage temperature of routine 8 types of

chemistry analytes in immediately cell-separated serum following storage at a designated temperature (4^0C and −20°C) for different periods and prolonged time i.e. 0, 7, 15, and 30 days using the standard guidelines for blood sample handling and separation.[10]

Aim of this study is to know the serum analytes stability at various time duration and temperature. Sometimes situation arise that demands re-estimation of particular analytes from the old sample itself though we always stick to standard protocols.

MATERIALS AND METHODS

This analytical study was approved by institutional ethical committee.

Subjects: 50 healthy and adult volunteers were studied after giving informed consent in Shri M. P. Shah government medical college, Jamnagar. There would be no financial or any other form of compensation given to the participants/volunteers. The study was carried out from April 2022 to March 2023.

Inclusion Criteria: Volunteers that were not suffering from infectious or chronic diseases, not to be on antibiotic treatment or any other medication for any other illness.

Exclusion Criteria: Volunteers that were suffering from any medical illness or on any medications were excluded from this study.

Laboratory Methods: Venous blood samples were obtained from 50 healthy adult individuals by two trained laboratory technicians with a puncture of the anterior bend of the elbow using a 21 G needle. Blood samples were collected in the morning. After the collection of blood samples were centrifuged at 2000 rpm for 10 minutes. After centrifugation serum analytes were assayed in primary tubes using a Techom fully auto analyzer. The residual serum was stored at 4^0C in the refrigerator and at -20^0C in the deep refrigerator. 8 aliquots of serum with a lid were stored at 4^0C and another 8 aliquots of serum were stored with a lid at -20^0C temperature.

Serum samples were separated into aliquots with lids. 6 aliquots of serum were stored at 4^0C and -20^0C. Therefore, 12 aliquots per patient were stored for 1, 7, 15, and 30 days to prevent the freeze-thawing of the samples in each analysis.

Following Biochemical parameters will be measured in a Fully Auto analyzer by using ongoing methods performing the clinical biochemistry laboratory.

Table 1: Analytes used in the study and methodology used for the analysis.

Analytes used	The methodology used for the Analysis
Urea	Berthlot
Creatinine	Mod. Jaffe's
Total Protein	Biuret
Total Albumin	B.C.G.
Sodium	ISE
Potassium	ISE
Chlorine	ISE
Bilirubin	Mod. Jandrassik Groff

Statistics

For each analyte and each storage condition, the mean of the results obtained from 50 samples was calculated. The percentage deviation in the value of the analyte was computed by subtracting values at baseline (To) from that at other periods (Tx) using the following formula:

Percentage deviation = [(Tx − To) / To] × 100

Subsequently for each condition (Day and Temperature) the p-values were obtained using the student's t-test. In the study p value <0.05 was considered statistically significant.

RESULTS

We evidenced the instability of Creatinine, Total Protein, Albumin, Sodium, Potassium, Chlorine, and Total, Direct, and Indirect Bilirubin. However, potential clinical impact significance was observed only for urea at T7 stored at -20^0C.

Table 2: Mean ±SD of urea from baseline values in blood sample exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Urea	Baseline Mean±SD	4 ^{0} C Mean±SD	% Change from Baseline	P Value	-20 ^{0} C Mean±SD	% Change from Baseline	P Value
Day 7	27.3±2.26	24.2±2.57	75.8 (↓)	<0.01	27.1±2.98	72.9(↓)	0.09
Day 15		19.7±2.70	80.3(↓)	<0.01	17.2±2.36	82.8 (↓)	<0.01
Day 30		33.1±2.63	66.9 (↑)	<0.01	29.1±2.81	70.9 (↑)	<0.01

Table 3: Mean ± SD of Creatinine from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Creatinine	Baseline	4 ^{0} C	%Change from Baseline	P Value	-20 ^{0} C	%Change from Baseline	P Value
Day 7	0.89±0.35	0.95±0.21	99.05 (↓)	0.29	0.96±0.21	99.04 (↓)	0.006
Day 15		0.96±0.21	99.04 (↓)	0.29	0.95±3.54	99.05 (↓)	0.23
Day 30		0.92±0.33	99.08 (↓)	0.67	1.05±0.19	98.95 (↓)	<0.01

Table 4: Mean ± SD of Total Protein from baseline values in blood sample exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Total Protein	Baseline	4 ^{0} C	%Change from Baseline	P Value	-20 ^{0} C	%Change from Baseline	P Value
Day 7	6.47±0.34	6.23±0.25	93.77 (↓)	0.0007	6.16±0.25	93.81 (↓)	<0.01
Day 15		5.96±0.32	94.04 (↓)	<0.01	6.03±0.43	93.97 (↓)	<0.01
Day 30		7.49±0.70	92.51 (↑)	<0.01	7.17±0.61	92.83 (↑)	<0.01

Table 5: Mean ±SD of Total Albumin from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Total Albumin	Baseline	4 ^{0} C	% Change from Baseline	P Value	-20 ^{0} C	% Change from Baseline	P Value
Day 7	4.19±0.40	3.87±0.18	96.13 (↓)	<0.01	3.91±0.22	96.09 (↓)	<0.01
Day 15		4.00±0.25	96.00 (↓)	0.02	3.95±0.68	96.05 (↓)	0.03
Day 30		4.00±0.35	96.00 (↓)	0.04	3.87±0.47	96.13 (↓)	0.004

Table 6: Mean ±SD of Sodium from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Sodium	Baseline	4 ^{0} C	% Change from Baseline	P Value	-20 ^{0} C	% Change from Baseline	P Value
Day 7	144.31±1.88	142.59±2.55	42.59 (↓)	<0.01	143.08±1.93	43.08 (↓)	0.001
Day 15		146.11±3.49	46.11 (↑)	0.03	144.83±2.14	44.83 (↑)	0.2
Day 30		153.38±1.93	53.38 (↑)	0.001	148.96±2.68	48.96 (↑)	<0.01

Table 7: Mean ±SD of Potassium from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Potassium	Baseline	4 ^{0} C	% Change from Baseline	P Value	-20 ^{0} C	% Change from Baseline	P Value
Day 7	4.59±0.40	4.63±0.46	95.37 (↑)	0.71	4.79±0.49	95.27 (↑)	0.33
Day 15		4.63±0.42	95.37 (↑)	0.72	4.73±0.43	95.21 (↑)	0.04
Day 30		5.00±0.49	95.00 (↑)	0.34	4.95±0.47	95.05 (↑)	0.0002

Table 8: Mean ±SD of Chlorine from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Chlorine	Baseline	4 ^{0} C	%Change from Baseline	P Value	-20 ^{0} C	% Change from Baseline	P Value
Day 7	108.23±1.39	106.69±1.91	6.69 (↓)	<0.01	107.31±1.46	7.31 (↓)	0.001
Day 15		109.59±2.62	9.59 (↑)	0.03	108.62±1.61	8.62 (↑)	0.22
Day 30		115.03±1.45	15.03 (↑)	0.001	111.72±2.01	11.72 (↑)	<0.01

Table 9: Mean ±SD of Total Bilirubin from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Total Bilirubin	Baseline	4 ^{0} C	% Change from Baseline	P Value	-20 ^{0} C	% Change from Baseline	P Value
Day 7	1.34±0.63	1.10±0.49	98.80 (↓)	0.20	1.24±0.50	98.76 (↓)	0.67
Day 15		1.00±0.52	99.00 (↓)	0.07	1.28±0.80	98.72 (↓)	0.70
Day 30		0.97±0.50	99.03 (↓)	0.67	1.00±0.46	99.00 (↓)	0.17

Table 10: Mean ±SD of Direct Bilirubin from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Direct Bilirubin	Baseline	4^0 C	% Change from Baseline	P Value	-20^0 C	% Change from Baseline	P Value
Day 7	0.42±0.22	0.36±0.20	99.64 (↓)	0.08	0.47±0.39	99.53 (↑)	0.35
Day 15		0.31±0.19	99.69 (↓)	0.06	0.41±0.26	99.59 (↓)	0.80
Day 30		0.34±0.28	99.66 (↓)	0.35	0.61±0.32	99.39 (↑)	0.43

Table 11: Mean ±SD of Indirect Bilirubin from baseline values in blood samples exposed to different days (Day 7, Day 15, Day 30) and different temperatures (4^0C and -20^0 C).

Indirect Bilirubin	Baseline	4^0 C	% Change from Baseline	P Value	-20^0 C	% Change from Baseline	P Value
Day 7	0.91±0.49	0.75±0.20	99.25 (↓)	0.44	0.71±0.39	99.29 (↓)	0.35
Day 15		0.72±0.43	99.28 (↓)	0.31	0.91±0.41	99.09 (↓)	0.80
Day 30		0.63±0.39	99.37 (↓)	0.28	0.65±0.41	99.35 (↓)	0.43

DISCUSSION

In this study, effect of storage at baseline and refrigeration (4^0C and -20^0C) for 7, 15, and 30 days were studied.

Vernekar and Jabanwar,[13] reported instability in urea and creatinine samples stored at -20^0C. Similar results were found in our study. In our study, we found a significant decrease in the concentration of urea for 7 and 15 days, and after 30 days concentration of urea increased. Urea at -20^0C is stable for 7 days. Some investigators have reported fair stability of many analytes in serum. A significant time-dependent increase in urea observed in this study was consistent with others previously reported by Boyanton and Blick,[14] Chudar *et al*.[11] and Vernakar and Jabannavar,[13] although substantial decrease of about 15% in levels has been reported by Brinc *et al*.,[19] Significant increase in both serum urea and creatinine levels with time in samples stored at -20^0C have been reported by Vernakar and Jabannavar.[13] This similar finding was however observed in only the samples stored at room and refrigerator temperatures.

No significant statistical or clinical differences were found in the concentration of creatinine in our study under the different storage conditions. Consistent with the previous study,[20] we did not detect any statistically or clinically significant change in creatinine levels. However, according to Boyanton and Blick,[14] the increase in serum creatinine levels after 24 h is due to serum cell contact at room temperature.[15] Similar results were found in our study. We found a non significant increased concentration of creatinine. We found a significant increase on day 7 at -20^0C temperature. In contrast with the 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.[13] Thus demonstrating 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.[21]

Statistical significant decrease in the concentration of total protein for both the temperature (4^0C and -20^0C) found in our study, but at day 30 we found a significantly increased concentration in total protein. A similar result was found by Boyanton and Blick,[15] and Cuhadar *et al*. who have reported an increase in total protein in their works.

Kachhawa *et al*.[2] noted no differences in serum protein (total protein and albumin) were detected at any of the three-time points compared to fresh samples for albumin and total protein in serum specimens. Moreover, our results are not similar to a previous study reporting clinically equivalent levels of total protein and albumin. In our study, we found a significant decrease in total albumin concentration for both the temperature and for all the duration (days 7, 15, and 30).

In agreement with the findings of Zhang *et al*.,[11] no clinically significant differences were found for sodium levels following different storage durations compared to fresh samples; however, our serum Na+ findings are not consistent with the findings of studies. We found a significant decrease in sodium concentration on day 7 for both temperatures. On day 15 it does not show any significant changes while on day 30 it shows a significant increase concentration of sodium.

Previous studies have demonstrated an increase in K+ after 24 h due to serum-cell contact at room temperature.[4,17] The increase in K+ after 24 h is most likely caused by malfunction of the Na+/K+ ATPase pump, resulting in diffusion of K+ from the erythrocytes driven by the intracellular–extracellular concentration gradient. Moreover, our results showed a clinically nonsignificant increase in K+ level.

Serum samples for bilirubin examination could not be stored at room temperature because of statistically significant variability. However, considering the p-value,

the sample for bilirubin determination could not be stored for more than 24 hours.

CONCLUSION

We recommend that samples be analyzed in the laboratory within 24 h of collection to ensure valid results. In addition, the turn-around time from sample drawing to reporting the analytical result would be shortened.

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