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Effect of Storage Time and Temperature on the Stability of Serum Analytes

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Abstract

Background

Determining the pattern of analytes stability with regard to time duration and temperature of storage is compulsory to maintain the precision and accuracy of measurements. Hence, this study aimed to assess the stability of 19 common serum analytes in relation to time length and storage temperatures.

Methods

Serum specimens from healthy adult volunteers were obtained, transferred into a single BD falcon tube, gently mixed. About 300µL of serum was taken and immediately analyzed to determine the concentration of 19 common serum analytes to use the results as a baseline value for the characterization of analytes stability. Then the serum aliquoted into 24 sterilized Nunc tubes (300µL in each) and six aliquots were stored in each storage temperature like 17°C, 2-8 °C, -20°C and -41°C. Then the aliquots analyzed in parallel on days 5, 10, 15, 20, 25, and 30.

Results

At 17°C, amylase (AMY), Urea (URE), total protein (TP), Creatinine (CREA) and Glucose (GLU) were stable until day 25 with <3% change of the starting value, while the rest analytes were very unstable throughout 30 days. At 2-8 °C, alkaline phosphatase (ALP), creatine phosphatase (CK-T), cholesterol (TC), AMY, LDL-cholesterol (LDL-c), TP, and GLU were stable until day 30 with ≤4% change of the initial value. At -20°C and -410C, AMY, pancreatic amylase (AM-P), LDH, TC, triglyceride (TG), GLU, PHO, and TP were stable for 30 days with ≤3.1% change from a baseline value. Novel findings: the following tests were stable at 170C for 25 days with the maximum change of urea <2%, glucose <3%, protein total <1% and amylase 0.0%

Conclusion

This study tried to evaluate the stability of serum biochemicals at four different storage temperatures concurrently. Each serum biochemical has inimitable stability characteristics in diverse storage temperatures. Therefore, clinical laboratories should store specimens using appropriate temperatures as soon as possible by considering the length of storage time.

Keywords: Serum, Biochemical analytes, Stability, Storage temperature

Introduction

The main challenge in clinical laboratories is analytes stability in serum/plasma if there is a delay in laboratory examination. Samples are commonly stored in the laboratory at different temperature levels until the laboratory examination done like15-25°C out of the refrigerator, 4-8°C (in the refrigerator) and in deep freezers (-20°C,-40°C, -70°C or -80°C) depending on the need of storage time. Therefore, temperature maintenance for sample storage is one of the critical parts of pre-analytical quality assurance and it plays a great role to maintain analytes stability or measurement precision. It is recommended that performing tests from freshly collected serum or plasma to maintain the integrity of results otherwise alteration in values might occur [1,2]. Factors like physiological and analytical, and age, sex, pregnancy, nutritional status, and postures can also consequence variabilities in lab results [3]. Studies reported short-term stability of common biochemical analytes in human serum specimens if the samples stored in the refrigerator (at 4 °C) and/or at room temperature (RT) [4, 5]. However, a deep freezer is required for prolonged storage and long-term stability of biochemical analytes in human samples at -20°C to -80°C temperature when compared to the storage temperature of 4°C or RT [6, 7, 8]. The temperature and length of storage are vital factors that may influence the outcome of serum biochemical examination. Data concerning the stability of serum analytes in relation to time duration and temperatures of storage in human serum samples are scarce in the Ethiopian setting.

Therefore, this study was carried out to address possible storage temperature for routine 19 types of serum biochemical

analytes using four different storage temperatures (17°C, 4°C, -20°C and -41°C) for 30 days prolonged time.

Methods

Study Setting

This prospective study was conducted from 24 April to 23 May 2020 at Hawassa University Comprehensive Specialized Hospital, Hawassa, Sidama regional state. Hawassa city is the capital city of the region and 275 km distant from the capital city of Ethiopia. This study was conducted in the clinical chemistry laboratory unit.

Sample Collection, Storage Temperature, and Measurements

Ten voluntary and physically healthy male individuals of similar age groups were selected and 5ml of blood was collected from each using an evacuated serum separator gel tube (SSGT) and kept 20-25minutes at room temperature in light protected place for complete clotting. The samples were centrifuged at room temperature (17OC) with 3000rpm for 10 minutes. Then serum was separated from cells by gel and clear serum of these three individuals was pipetted by Pasteur pipette, transferred to 14ml containing BD falcon tube, and gently mixed before doing aliquots. Immediately 300µl of serum was taken and immediately analyzed to determine the concentration of 19 common serum analytes by Cobas 600Integra series analyzer from Roche Diagnostics (Germany) to use results as a baseline value. Then serum was equal aliquoted in 24 parts with a volume of 300µl in each sterile plastic tube and tightly closed with a lid. Also, six aliquots were stored in each storage temperature of 17°C, 2-8 °C, -20°C, and -41°C. Then one aliquot from each storage temperature was taken in every time interval, kept in room temperature for 30 minutes before analysis, and then analyzed in parallel on the day: 5, 10, 15, 20, 25, and 30. To avoid instrument, methods and reagent variability effect, the study used a single analyzer and the same batch reagents in the follow-up measurements as those measured at the baseline. These19 serum analytes were aspartate aminotransferase(AST), alanine aminotransferase(ALT), alkaline phosphatase(ALP), creatine kinase (CK-T), lactate dehydrogenase (LDH), total amylase(AMY), pancreatic amylase(AM-P), lipase(LIP), urea(URE), creatinine(CREA), glucose(GLU), total protein(TP), total cholesterol(TC), HDL-cholesterol(HDL-c), LDL-cholesterol (LDL-c), direct bilirubin (BILD), total bilirubin(BIL-T), triglyceride (TG) and phosphates (PHO).

Outcome Assessment and Statistical Analysis

As stated above, this study assessed the levels and trends of 19 serum biochemical analytes, at baseline (time 0) of the study and repeatedly measured on days 5, 10, 15, 20, 25, and 30 after the baseline. Normality and homogeneity of variance assumptions of the distribution of analytes were checked by assessing histograms and normal probability plots using the Shapiro-Wilk test. Means Student t-test was used to find the effect of aliquots storage temperature and length of storage time on its analytes concentration. The mean difference of analytes between storage temperatures was considered significant at $p<0.05$. The analyte concentration at zero time was considered as a control. The change of each analyte from baseline (time 0) for different storage temperatures was calculated as percent change as follows: Percent change of analyte from baseline at Y = concentration at $[(Xn-X0)/X0] \times 100$. Where, Y is storage temperature (17°C, 4°C, -20°C and -41°C); Xn is the concentration of analyte at the specific time of aliquots analysis (day 5, 10, 15, 20, 25 and 30); X0 is the concentration of analyte at baseline (time 0). The imprecision limit for each analyte also was determined by multiplying coefficient variation (CV) with 0.5 or (% CV x0.5). Statistically significant changes in between storage temperatures, and time intervals also was determined for each analyte by ANOVA paired way analysis. Post-hoc analysis was assessed with a Bonferroni adjustment to ensuing a significance level at $P < 0.008$ (0.05/6 = 0.008) was set for each analyte regarding time interval and $p<0.0125$ (0.05/4) was set for each analyte regarding storage temperature.

Results

The Pattern of Analytes Stability at 170c

At170C storage temperature, baseline value of AST was 52.1U/L and on day 5, it was slightly decreased to 49.1U/L, on day 10 it also decreased to 43.8U/L, but on day 25 and day 30, it was significantly decreased to 32.4U/L and 37.7U/L, respectively. AMY was stable until day25 without any change (76U/L) in all storage temperatures, but on day 30, it was slightly increased to79U/L (3.9%). LDH significantly decreased from the baseline value (286U/L) to 264 U/L, 257 U/L, 230U/L, and 277U/L on days 10, 15, 25, and 30, respectively. In addition, the overall percent change of analyte was 4.4% to 22.3% for LDL-c, 6.1% to 32% for TG, and 3.9% to 40.9% for PHO. However, a significant decrease was observed from baseline in AST (-37.8% at day 25), ALT (-82.9% at day 25), BIL-D (-70.9% at day 30%), BIL-T (-65.8% at day 30) and HDL (-29.8% at day 25) (Table-1).

Analyte	Concentrations of analytes and days of run							Change from baseline		% Change from baseline									
	Baseline	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30

AST (U/L)	52.1	49.1	43.8	41.1	42.8	32.4	37.7	-3	-8.3	-11	-9.3	-19.7	-14.4	-5.8	-15.9	-21.1	-17.9	-37.8	-27.6
ALT (U/L)	35	21.2	15.1	12.2	20.5	6	12.6	-13.8	-19.9	-22.8	-14.5	-29	-22.4	-39.4	-56.9	-65.1	-41.4	-82.9	-64
ALP (U/L)	101	104	105	108	111	108	114	3	4	7	10	7	13	3	4	6.9	9.9	6.9	12.9
AMY (U/L)	76	76	76	76	76	76	79	0	0	0	0	0	3	0	0	0	0	0	3.9
AM-P (U/L)	32.4	32.6	34.3	34.8	34.5	36.5	36.7	0.2	1.9	2.4	2.1	4.1	4.3	0.6	5.9	7.4	6.5	12.7	13.3
LDH (U/L)	286	279	264	257	276	230	277	-7	-22	-29	-10	-56	-9	-2.4	-7.7	-10.1	-3.5	-19.6	-3.1
CK-T (U/L)	464	384	331	300	431	224	390	-80	-133	-164	-33	-240	-74	-17.2	-28.7	-35.3	-7.1	-51.7	-15.9
LIP (U/L)	28.2	26.3	25.6	23.8	25.3	20.8	24.6	-1.9	-2.6	-4.4	-2.9	-7.4	-3.6	-6.7	-9.2	-15.6	-10.3	-26.2	-12.8
TC (mg/dL)	119.9	120.1	120.4	119.5	124.5	123.6	127.3	0.2	0.5	-0.4	4.6	3.7	7.4	0.2	0.4	-0.3	3.8	3.1	6.2
HDL-c (Mg/dL)	35.9	32.4	31	29	29.5	25.2	28	-3.5	-4.9	-6.9	-6.4	-10.7	-7.9	-9.7	-13.6	-19.2	-17.8	-29.8	-22
LDL-c (mg/dL)	52.4	54.7	57.1	59.4	60.8	62.5	64.1	2.3	4.7	7	8.4	10.1	11.7	4.4	9	13.4	16	19.3	22.3
TG (mg/dL)	161.4	171.3	181.8	189.2	189.7	213.3	210.7	9.9	20.4	27.8	28.3	51.9	49.3	6.1	12.6	17.2	17.5	32	30.5
BIL-D (mg/dL)	0.44	0.311	0.259	0.223	0.156	0.182	0.128	-0.13	-0.18	-0.22	-0.28	-0.26	-0.31	-29.3	-41.1	-49.3	-64.5	-58.6	-70.9
BIL-T (mg/dL)	0.85	0.767	0.694	0.556	0.424	0.435	0.291	-0.08	-0.16	-0.29	-0.43	-0.42	-0.56	-9.8	-18.4	-34.6	-50.1	-48.8	-65.8
UREA (mg/dl)	52	52.9	51.9	52.5	53.1	52.9	54.9	0.9	-0.1	0.5	1.1	0.9	2.9	1.7	-0.2	1	2.1	1.7	5.6
CREA (mg/dL)	1.38	1.38	1.41	1.33	1.36	1.34	1.38	0	0.03	-0.05	-0.02	-0.04	0	0	2.2	-3.6	-1.4	-2.9	0
GLU (mg/dL)	117.2	116	113.8	113.9	117.9	114.7	122.2	-1.2	-3.4	-3.3	0.7	-2.5	5	-1	-2.9	-2.8	0.6	-2.1	4.3
PHO (mmol/L)	1.27	1.32	1.41	1.53	1.56	1.76	1.79	0.05	0.14	0.26	0.29	0.49	0.52	3.9	11	20.5	22.8	38.6	40.9

TP (mg /dL)	5.4	5.44	5.39	5.44	5.42	5.42	5.74	0.04	-0.01	0.04	0.02	0.02	0.34	0.74	-0.2	0.7	0.37	-0.37	6.3

Table 1: Percent change in analytes concentration from baseline among aliquots stored at 170C.

The pattern of analytes stability at 2-8OC

At 2-80C storage temperature, the following analytes indicated change between the storage time: for example, TP (1.9% at day 30), GLU (-1.6% at day 5 and 1.6% at day 20), CREA (3.6% at day 10) and LDL-c (- 1.5% at day 25). In addition, slight increase was observed in ALP (4% at day 30), AMY (-1.3% at day 25 and1.3% at day 10), AM-P (4% at day 10) from baseline value (Table-2).

Analyte	**Concentrations of analytes and days of run**							**Change from baseline**	**% change from baseline**										
	Baseline	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
AST (U/L)	52.1	53	51.9	51.3	50.6	49.3	46.1	0.9	-0.2	-0.8	-1.5	-2.8	-6	1.7	-0.4	-1.5	-2.9	-5.4	-11
ALT(U/L)	35	33.6	28.3	26.9	24.8	24.4	18.8	-1.4	-6.7	-8.1	-10	-10.6	-16.2	-4	-19.1	-23.1	-29.1	-30.3	-46.3
ALP (U/L)	101	104	102	102	103	101	105	3	1	1	2	0	4	3	1	1	2	0	4
AMY (U/L)	76	76	77	76	76	75	76	0	1	0	0	-1	0	0	1.3	0	0	-1.3	0
AM-P (U/L)	32.4	32.3	33.7	33.2	32.5	32.6	33.3	-0.1	1.3	0.8	0.1	0.2	0.9	-0.3	4	2.5	0.3	0.6	2.8
LDH (U/L)	286	271	242	233	229	214	204	-15	-44	-53	-57	-72	-82	-5.2	-15.4	-18.5	-19.9	-25.2	-28.7
CK-T (U/L)	464	460	455	461	465	453	448	-4	-9	-3	1	-11	-16	-0.9	-1.9	-0.6	0.2	-2.4	-3.4
LIP (U/L)	28.2	28	27	27.8	25.9	26.6	25.2	-0.2	-1.2	-0.4	-2.3	-1.6	-3	-0.7	-4.3	-1.4	-8.2	-5.7	-10.6
TC (mg /dL)	119.9	121.6	121.7	121.1	123.4	122.2	123.3	1.7	1.8	1.2	3.5	2.3	3.4	1.4	1.5	1	2.9	1.9	2.8
HDL-c (Mg /dL)	35.9	34.2	33.9	33.2	32.8	33.4	31.5	-1.7	-2	-2.7	-3.1	-2.5	-4.4	-4.7	-5.6	-7.5	-8.6	-7	-12.3
LDL-c (mg /dL)	52.4	50.3	52.4	50.9	51.1	51.6	53.2	-2.1	0	-1.5	-1.3	-0.8	0.8	-4	0	-2.9	-2.5	-1.5	1.5
TG (mg /dL)	161.4	162.8	171	166.9	171.8	170.5	174.5	1.4	9.6	5.5	10.4	9.1	13.1	0.9	5.9	3.4	6.4	5.6	8.1

BIL-D (mg/dL)	0.44	0.403	0.349	0.319	0.299	0.309	0.259	-0.04	-0.09	-0.12	-0.14	-0.13	-0.18	-8.4	-20.7	-27.5	-32	-29.8	-41.1
BIL-T (mg/dL)	0.85	0.8	0.8	0.761	0.785	0.75	0.67	-0.05	-0.05	-0.09	-0.06	-0.1	-0.18	-5.9	-5.9	-10.5	-7.6	-12.1	-21.3
URE (mg/dl)	52	53.6	52.3	52.6	52.4	52.2	53.9	1.6	0.3	0.6	0.4	0.2	1.9	3	0.6	1.2	0.8	0.4	3.7
CREA (mg/dL)	1.38	1.39	1.43	1.38	1.36	1.36	1.39	0.01	0.05	0	-0.02	-0.02	0.01	0.7	3.6	0	-1.4	-1.4	0.7
GLU (mg/dL)	117.2	115.3	117.7	116.6	119.1	116.5	116.8	-1.9	0.5	-0.6	1.9	-0.7	-0.4	-1.6	0.4	-0.5	1.6	-0.6	-0.3
PHO (mmol/L)	1.27	1.3	1.31	1.36	1.35	1.34	1.39	0.03	0.04	0.09	0.08	0.07	0.12	2.4	3.1	7.1	6.3	5.5	9.4
TP (mg/dL)	5.4	5.38	5.47	5.47	5.47	5.4	5.5	-0.02	0.07	0.07	0.07	0	0.1	-0.4	1.3	1.3	1.3	0	1.9

Table 2: Percent change in analytes concentration from baseline among aliquots stored at 2-80C.

The pattern of analytes stability at -20OC

Among specimens stored at-200C freezer, TP, PHO, GLU, CREA, BIL-T, TG, LDL-c, HDL-c, TC, LIP, AM-P, AMY, and ALP indicated <5% change throughout 30 days from baseline, whereas ALT and CK showed high instability. In addition, BIL-D and AST also were unstable throughout 30 days duration at -20OC temperature (Table-3).

Analyte	**Concentrations of analytes and days of run**							**Change from baseline**	**% change from baseline**										
	Baseline	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
AST (U/L)	52.1	50.7	51.1	49.7	49.2	49.4	48.8	-1.4	-1	-2.4	-2.9	-2.7	-3.3	-2.7	-1.9	-4.6	-5.6	-5.2	-6.3
ALT(U/L)	35	32.9	32.4	31.8	29.7	28.5	28.3	-2.1	-2.6	-3.2	-5.3	-6.5	-6.7	-6	-7.4	-9.1	-15.1	-18.6	-19.1
ALP (U/L)	101	103	104	104	101	103	102	2	3	3	0	2	1	2	3	3	0	2	1
AMY(U/L)	76	75	76	76	76	76	76	-1	0	0	0	0	0	-1.3	0	0	0	0	0
AM-P (U/L)	32.4	32.3	32.4	32.4	32.2	32.4	32.5	-0.1	0	0	-0.2	0	0.1	-0.3	0	0	-0.6	0	0.3

LDH (U/L)	286	280	279	281	279	283	278	-6	-7	-5	-7	-3	-8	-2.1	-2.4	-1.7	-2.4	-1	-2.8
CK-T (U/L)	464	444	439	433	423	431	415	-20	-25	-31	-41	-33	-49	-4.3	-5.4	-6.7	-8.8	-7.1	-10.6
LIP (U/L)	28.2	27.1	26.8	28	27.9	28.5	27.1	-1.1	-1.4	-0.2	-0.3	0.3	-1.1	-3.9	-4.9	-0.7	-1.1	1.1	-3.9
TC (mg/dL)	119.9	120.2	119.7	119	119.7	118.8	122.5	0.3	-0.2	-0.9	-0.2	-1.1	2.6	0.3	-0.2	-0.8	-0.2	-0.9	2.2
HDL-c (Mg/dL)	35.9	35.4	35.3	35.4	35.3	35.9	35.4	-0.5	-0.6	-0.5	-0.06	0	-0.5	-1.4	-1.7	-1.4	-1.7	0	-1.4
LDL-c (mg/dL)	52.4	50.6	51.1	50.6	51.4	50.1	50	-1.8	-1.3	-1.8	-1	-2.3	-2.4	-3.4	-2.5	-3.4	-1.9	-4.4	-4.6
TG (mg/dL)	161.4	162.8	161.4	159.2	165.3	162.4	161.7	1.4	0	-2.2	3.9	1	0.3	0.9	0	-1.4	2.4	0.6	0.18
BIL-D (mg/dL)	0.44	0.43	0.416	0.424	0.419	0.439	0.435	-0.01	-0.02	-0.02	-0.02	0	-0.01	-2.3	-5.5	-3.6	-4.8	-0.2	-1.1
BIL-T (mg/dL)	0.85	0.841	0.81	0.858	0.841	0.85	0.82	-0.01	-0.04	0.01	-0.01	0	-0.03	-1.1	-4.5	0.9	-1.1	0.5	-3.2
URE (mg/dL)	52	52.9	50.5	50.5	51	51.4	51.5	0.9	-1.5	-1.5	-1	-0.6	-0.5	1.7	-2.9	-2.9	-1.9	-1.2	-1
CREA (mg/dL)	1.38	1.41	1.37	1.4	1.4	1.4	1.34	0.03	-0.01	0.02	0.02	0.02	-0.04	2.2	-0.7	1.4	1.4	1.4	-2.9
GLU (mg/dL)	117.2	114.9	117.9	116.2	116.3	114.8	116.7	-2.3	0.7	-1	-0.9	-2.4	-0.5	-2	0.6	-0.9	-0.8	-2	-0.4
PHO (mmol/L)	1.27	1.28	1.29	1.26	1.28	1.28	1.25	0.01	0.02	-0.01	0.01	0.01	-0.02	0.8	1.6	-0.8	0.8	0.8	-1.6
TP (mg/dL)	5.4	5.43	5.37	5.36	5.48	5.38	5.4	0.03	-0.03	-0.04	0.08	-0.02	0	0.6	-0.6	-0.7	1.5	-0.4	0

Table 3: Percent change in analytes concentration from baseline among aliquots stored at -200C.

The pattern of analytes stability at -41OC

Among aliquots stored at -410C temperature in the freezer, ALP, AMY, AM-P, TC, LDH, HDL-c, TG, URE, GLU, PHO and TP have indicated a change <4% from baseline. Besides, CREA, BIL-T, and LDL-c analytes indicated a change of less than or equal to 5%. However, ALT was decreased >16% after day 15 and CK-T decreased > 7% after day 15 when compared with baseline value(Table-4).

Analyte	Concentrations of analyte and days of run	Change from	% change fro	

								baseline	mbaseline										
Baseline	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	
AST (U/L)	52.1	52.2	51.1	50	49.8	50.2	49.9	0.1	-1	-2.1	-2.3	-1.9	-2.2	0.2	-1.9	-4	-4.4	-3.6	-4.2
ALT(U/L)	35	34.2	34.1	32.4	29.3	29.6	25.4	-0.8	-0.9	-2.6	-5.7	-5.4	-9.6	-2.3	-2.6	-7.4	-16.3	-15.4	-27.4
ALP (U/L)	101	101	102	102	104	104	103	0	1	1	3	3	2	0	1	1	3	3	2
AMY(U/L)	76	76	76	76	75	76	76	0	0	0	-1	0	0	0	0	0	-1.3	0	0
AM-P (U/L)	32.4	32.7	32.9	32.6	32.7	32.8	32.8	0.3	0.5	0.2	0.3	0.4	0.4	0.9	1.5	0.6	0.9	1.2	1.2
LDH (U/L)	286	290	290	279	280	285	283	4	4	-7	-6	-1	-3	1.4	1.4	-2.4	-2.1	-0.3	-1
CK-T (U/L)	464	456	455	439	430	438	410	-8	-9	-25	-34	-26	-54	-1.7	-1.9	-5.4	-7.3	-5.6	-11.6
LIP (U/L)	28.2	28.1	27.9	27.6	27.9	29.9	27.1	-0.1	-0.3	-0.6	-0.3	1.7	-1.1	-0.4	-1.1	-2.1	-1.1	6	-3.9
TC (mg/dL)	119.9	122	118.7	118.6	120.1	119.5	118.2	2.1	-1.2	-1.3	0.2	-0.4	-1.7	1.8	-1	-1.1	0.2	-0.3	-1.4
HDL-c (Mg/dL)	35.9	36.2	36	35.8	35.6	35.8	35	0.3	0.1	-0.1	-0.3	-0.1	-0.9	0.8	0.3	-0.3	-0.8	-0.3	-2.5
LDL-c (mg/dL)	52.4	52	50.8	50.4	50.4	49.9	47.6	-0.4	-1.6	-2	-2	-2.5	-4.8	-0.8	-3.1	-3.8	-3.8	-4.8	-9.2
TG (mg/dL)	161.4	163.1	164.1	163.4	161.6	164.2	161.7	1.7	2.7	2	0.2	2.8	0.3	1.1	1.7	1.2	0.1	1.7	0.2
BIL-D (mg/dL)	0.44	0.439	0.424	0.425	0.42	0.431	0.411	0	-0.02	-0.02	-0.02	-0.01	-0.03	-0.2	-3.6	-3.4	-4.5	-2	-6.6
BIL-T (mg/dL)	0.85	0.85	0.816	0.823	0.843	0.843	0.843	0	-0.03	-0.03	-0.01	-0.01	-0.01	0	-4	-3.2	-0.8	-0.8	-0.8
URE (mg/dl)	52	51.5	51	52.2	51.3	51.3	50.4	-0.5	-1	0.2	-0.7	-0.7	-1.6	-1	-1.9	0.4	-1.3	-1.3	-3.1
CREA (mg/dL)	1.38	1.38	1.44	1.37	1.33	1.39	1.37	0	0.06	-0.01	-0.05	0.01	-0.01	0	4.3	-0.7	-3.6	0.7	-0.7
GLU	117.2	115.3	118.2	116.4	115.5	115.8	116.4	-1.9	1	-0.8	-1.7	-1.4	-0.8	-1.6	0.9	-0.7	-1.5	-1.2	-0.7

ALP(U/L)	3	4	6.9	9.9	6.9	12.9	3	1	1	2	0	4	2	3	3	0	2	1	0	1	1	3	3	2
AMY(U/L)	0	0	0	0	0	3.9	0	1.3	0	0	-1.3	0	-1.3	0	0	0	0	0	0	0	0	-1.3	0	0
AM-P(U/L)	0.6	5.9	7.4	6.5	12.7	13.3	-0.3	4	2.5	0.3	0.6	2.8	-0.3	0	0	-0.6	0	0.3	0.9	1.5	0.6	0.9	1.2	1.2
LDH(U/L)	-2.4	-7.7	-10.1	-3.5	-19.6	-3.1	-5.2	-15.4	-18.5	-19.9	-25.2	-28.7	-2.1	-2.4	-1.7	-2.4	-1	-2.8	1.4	1.4	-2.4	-2.1	-0.3	-1
CK-T(U/L)	-17.2	-28.7	-35.3	-7.1	-51.7	-15.9	-0.9	-1.9	-0.6	0.2	-2.4	-3.4	-4.3	-5.4	-6.7	-8.8	-7.1	-10.6	-1.7	-1.9	-5.4	-7.3	-5.6	-11.6
LIP(U/L)	-6.7	-9.2	-15.6	-10.3	-26.2	-12.8	-0.7	-4.3	-1.4	-8.2	-5.7	-10.6	-3.9	-4.9	-0.7	-1.1	1.1	-3.9	-0.4	-1.1	-2.1	-1.1	6	-3.9
TC(mg/dL)	0.2	0.4	-0.3	3.8	3.1	6.2	1.4	1.5	1	2.9	1.9	2.8	0.3	-0.2	-0.8	-0.2	-0.9	2.2	1.8	-1	-1.1	0.2	-0.3	-1.4
HDL-c(Mg/dL)	-9.7	-13.6	-19.2	-17.8	-29.8	-22	-4.7	-5.6	-7.5	-8.6	-7	-12.3	-1.4	-1.7	-1.4	-1.7	0	-1.4	0.8	0.3	-0.3	-0.8	-0.3	-2.5
LDL-c(mg/dL)	4.4	9	13.4	16	19.3	22.3	-4	0	-2.9	-2.5	-1.5	1.5	-3.4	-2.5	-3.4	-1.9	-4.4	-4.6	-0.8	-3.1	-3.8	-3.8	-4.8	-9.2
TG(mg/dL)	6.1	12.6	17.2	17.5	32	30.5	0.9	5.9	3.4	6.4	5.6	8.1	0.9	0	-1.4	2.4	0.6	0.18	1.1	1.7	1.2	0.1	1.7	0.2
BIL-D(mg/	-29.3	-41.1	-49.3	-64.5	-58.6	-70.9	-8.4	-20.7	-27.5	-32	-29.8	-41.1	-2.3	-5.5	-3.6	-4.8	-0.2	-1.1	-0.2	-3.6	-3.4	-4.5	-2	-6.6

							nanbias	**nanbias**	**nanbias**																	
	5thday	10thday	15thday	20thday	25thday	30thday	5thday	10thday	15thday	20thday	25thday	30thday	5thday	10thday	15thday	20thday	25thday	30thday	5thday	10thday	15thday	20thday	25thday	30thday	
AST	-5.8	-15.9	-21.1	-17.9	-37.8	-27.6	1.7	-0.4	-1.5	-2.9	-5.4	-11	-2.7	-1.9	-4.6	-5.6	-5.2	-6.3	0.2	-1.9	-4	-4.4	-3.6	-4.2	5.15
ALT(U/L)	-39.4	-56.9	-65.1	-41.4	-82.9	-64	-4	-19.1	-23.1	-29.1	-30.3	-46.3	-6	-7.4	-9.1	-15.1	-18.6	-19.1	-2.3	-2.6	-7.4	-16.3	-15.4	-27.4	15.19
ALP(U/L)	3	4	6.9	9.9	6.9	12.9	3	1	1	2	0	4	2	3	3	0	2	1	0	1	1	3	3	2	1.53
AMY(U/L)	0	0	0	0	0	3.9	0	1.3	0	0	-1.3	0	-1.3	0	0	0	0	0	0	0	0	-1.3	0	0	0.48
AM-P(U/L)	0.6	5.9	7.4	6.5	12.7	13.3	-0.3	4	2.5	0.3	0.6	2.8	-0.3	0	0	-0.6	0	0.3	0.9	1.5	0.6	0.9	1.2	1.2	1.87
LDH(U/L)	-2.4	-7.7	-10.1	-3.5	-19.6	-3.1	-5.2	-15.4	-18.5	-19.9	-25.2	-28.7	-2.1	-2.4	-1.7	-2.4	-1	-2.8	1.4	1.4	-2.4	-2.1	-0.3	-1	4.72
CK-T(U/L)	-17.2	-28.7	-35.3	-7.1	-51.7	-15.9	-0.9	-1.9	-0.6	0.2	-2.4	-3.4	-4.3	-5.4	-6.7	-8.8	-7.1	-10.6	-1.7	-1.9	-5.4	-7.3	-5.6	-11.6	6.8
LIP(U/L)	-6.7	-9.2	-15.6	-10.3	-26.2	-12.8	-0.7	-4.3	-1.4	-8.2	-5.7	-10.6	-3.9	-4.9	-0.7	-1.1	1.1	-3.9	-0.4	-1.1	-2.1	-1.1	6	-3.9	3.45
TC(mg/dL)	0.2	0.4	-0.3	3.8	3.1	6.2	1.4	1.5	1	2.9	1.9	2.8	0.3	-0.2	-0.8	-0.2	-0.9	2.2	1.8	-1	-1.1	0.2	-0.3	-1.4	0.9
HDL-c(Mg/dL)	-9.7	-13.6	-19.2	-17.8	-29.8	-22	-4.7	-5.6	-7.5	-8.6	-7	-12.3	-1.4	-1.7	-1.4	-1.7	0	-1.4	0.8	0.3	-0.3	-0.8	-0.3	-2.5	4.39

Imprecision limit of each analyte = 0.5 ×% CV (coefficient variation); percent change= [(Xn- X0)/ X0] x100, where Xn is a concentration of analyte at n day run, X0 is a concentration of analyte at baseline

Change of analytes at 30 days of storage

AST, showed a signi icance differences between the storage temperatures of 17 oC vs.2-8oC and 17 oC vs.-41oC. ALT and ALP also indicated significant variation beteween temperature 17 oC vs.2-8oC, 17 oC vs.-20oC and 17 oC vs.-41oC. While, BIL-D was significantly differ beteween17vs.2-8oC, 17vs.-20oC, 2-8 oCvs.-20oC, 17vs.-41oC and 2-8vs.-41oC (Table-7).

In Post-hoc analysis except AMY, CREA, and GLU; the rest analytes indicated significance differences between the storage temperatures (P-value <0.0125), while all parameters did not show any significant difference regarding storage time within a group (p-value >0.008).

Parameter	17 oC vs.2-8oC	17 oC vs.-20oC	17 oC vs.-41oC	2-8 oC vs.-20oC	2-8 oC vs.-41oC	-20 oC vs.-41oC
AST (U/L)	41.1vs.50.4**	41.1vs.50.1**	41.1vs.50.1**	50.4vs.50.1	50.4vs.50.1	50.1vs.50.1
ALT(U/L)	14.6vs.26.1**	14.6vs.31.2***	14.6vs.30.8***	26.1vs31.2*	26.1vs.30.8	31.2vs.30.8
ALP(U/L)	108.3vs.102.8**	108.3vs.102.6**	108.3vs.102.7**	102.8v102.6	102.8vs.102.7	102.6vs.102.7
AMY(U/L)	76.5vs.76	76.5vs.75.8	76.5vs.75.8	76vs.75.8	76vs.75.8	75.8vs.75.8
AM-P(U/L)	34.9vs.32.9*	34.9vs.32.37**	34.9vs.32.7**	32.9vs.32.37*	32.9vs.32.7	32.4vs.32.7***
LDH(U/L)	263.8 vs.232.2*	263.8 vs.281*	263.8vs.284*	232.2vs.281***	232.2vs.284***	281vs.284
CK-T(U/L)	343.3vs.457**	343.3vs.435.6**	343.3vs.438*	457vs.435.6*	457vs.438*	435.6vs.438
LIP(U/L)	24.4vs.26.7*	24.4vs.27.6**	24.4vs.28.1**	26.7vs.27.6	26.7vs.28.1*	27.6vs.28.1
TC (mg/dL)	122.6vs.122.2	122.6vs.119.9	122.6vs.119.5	122.2vs.119.9**	122.2vs.119.5**	119.9vs.119.5
HDL-c(mg/dL)	29.2vs.33.2**	29.2vs.35.5***	29.2vs.35.7***	33.2vs.35.5***	33.2vs.35.7***	35.5vs.35.7
LDL-c(mg/dL)	59.8vs51.6***	59.8vs50.9***	59.8vs.50.2***	51.6vs50.9	51.6vs.50.2	50.9vs.50.2
TG(mg/dL)	192.7vs.169.6**	192.7vs.162***	192.7vs.163**	169.6vs.162**	169.6vs.163**	162vs.163
BIL-D(mg/dL)	0.21vs.0.32**	0.21vs.0.43***	0.21vs0.42***	0.32vs.0.43***	0.32vs0.42**	0.43vs0.42
BIL-T(mg/dL)	0.53vs.0.76*	0.53vs.0.84**	0.53vs.0.836**	0.76vs.0.84**	0.76vs.0.836**	0.84vs.0.836
URE(mg/dL)	53vs.52.3**	53vs.51.4**	53vs.51.3**	52.8vs.51.4**	52.8vs.51.3**	51.4vs.51.3
CREA(mg/dL)	1.37vs.1.38	1.37vs.1.38	1.37vs.1.38	1.38vs.1.38	1.38vs.1.38	1.38vs.1.38
GLU(mg/dL)	116.4vs.117	116.4vs.116.3	116.4vs.116.3	117vs116.3	117vs.116.3	116.3vs.116.3
PHO(mmol/L)	1.56vs1.56*	1.56vs1.27**	1.56vs.1.28	1.34vs1.27***	1.34vs.1.28**	1.27vs.1.28
TP ((mg/dL)	5.47vs.5.45	5.47vs.5.4	5.47vs.5.39	5.44vs.5.4	5.44vs.5.39*	5.4vs.5.39

Table 7: The overall mean value of analytes between storage temperatures and significances level.

*, p<0.05, **, p<0.01, ***, p<0.0001

Discussion

This study used 17°C, 2-8°C, -20°C and -41°C storage temperatures for storing aliquots intended for 30 days with the strict following of temperaturefluctuation using digital indicators. To indicate the effects of specimen storage period and temperature, primarily the concentration of 19 analytes was determined immediately after the serum has been separated and mixed the results were used as a baseline value to address the stability pattern of each analyte from baseline value.

In the present study AST was decreased to -5.8% on day 5 at 170C, -2.9% on day 20 at 2-80C, - 4.6% on day 15, -200C and -4.2% on day 30 at -410C of the storage temperatures. However, different studies highlighted the percent change of AST in different times and temperatures level of specimen storage: for example, the study conducted by Cray et al. indicated 3.8% of AST change on 7 days of specimen storage at 2-80C, 1.8% (frost-free freezer) and 1.1% (frost non-free freezer) of AST change on 30 days among the specimen stored at -200C[9]. In addition, the study reported by Shimizu et al. indicated -15.3% of AST changes among the specimen stored for 28 days at 2-80C and -4.8% changes, which stored at -200C for 14 days [10]. Moreover, AST decreased -7.1% at room temperature on day 7 and which increased 7.1% at 40C on day 7[11]. Day to day performance issues of the instrument, the nature of the enzyme itself, onboard duration of reagents, and minor alteration in temperature level might be plausible factors for the variations.

In this study, LDH was stable for the first 5 days at 170C, while it was stable for 30 days on aliquots stored at -200C and -410C with <3% change. The finding was inconsistent with the reports of several studies that indicated at least14 days' stability when the specimen stored at 250C and -300C [10] and 7 days stability at room temperature with 3.1% increase and 7 days stability at 40C with 1.8% decrease from the baseline [11]. The molecular nature and variability in the stability of LDH isoenzymes might cause significant instability with time length and temperature of specimen storage.

Friedman et al. reported 3 months of prolonged stability of CK-T isoenzymes activity at the storage temperature of 40C [12]. In similar, this study indicated the stability of CK-T activity for 30 days at 2-80C with a maximum decreasing of 3.4%. In contrast, the study indicated a more than 10% decrease in CK activity at 2-80C on day 7 [9]. In addition, Ikeda et al. also reported that the decreasing activity of CK at 40C and -200C [13]. It is difficult to clarify the disparities of CK activity in different temperatures, however, its three isoenzymes might not have a similar stability rate at different temperatures and time lengths of specimen storage.

Kachhawa et al. reported a significant decrease of AST, ALT, and ALP activity on day 30 from specimens stored at -20°C [14]. In line with the report of Kachhawa et al., the current study indicated a significant decrease among specimen stored at -20°C for 30 days; except for ALP (because ALP was stable and its maximum change was 1.3% during 30 days). In addition, the report of several studies indicated the instability of ALT activity at room temperature and -20°C and its comparative stability when the specimens stored at 4°C [13, 15]. However, in this study, ALP was stable for 30 days at 2-8°C with a maximum change of 4%.

One study revealed the stability of AMY and HDL-c until day56 at -20°C,-30°C, 4°C, and 25°C [10]. This in line with the finding of the current study except for HDL-c result of aliquots stored at17°Cand 2-8°C [10].

In this study, BIL-T was decreased from 5.9% to 21.3% within 30 days among aliquots stored at 2-8°C and similarly Tambse et al. and other studies revealed that the reduction of BIL-T staring from hours at 2-8°C [16, 17, 18]. Conversely, Shimizu et al. reported the stability of BIL-T with minor change at 4°C [10]. Serum separation time, level of hemolysis, and other substances may affect the stability bilirubin [19]. Further, BIL-T was stable for 1 month at -20°C and -41°C with a maximum 4.5% decrease in the current study and this finding in line with the study reported from aliquots stored at -20°C and -30°C [10].

Limitation of the Study

First, the lack of -70 to -80°C freezer made difficulties to assess the trend of serum biochemicals stability at -70°C and -80°C. Second, the study used 30 days of storage time, however variable stability rate might be observed if the specimens stored for several months to years. Third Irrespective of the described limits, this study ultimately awakes clinical laboratory personnel's concerning the length of specimen storage and temperature-based stability of analytes.

Conclusion

AST showed variation ≤2.5% among the specimens stored for 20 days at 2-80C and 10 days at -410C, whereas AST also indicated ≤ a 5% change on day 20 at 2-80C, on day 15 at -200C and day 30 at -410C on day 10. ALT was decreased <3% at -410C and on day 5 at 2-80C with 4% decrease. ALP showed a maximum of 4% on day 10 at 170C and day 30 at 2-80C. While on day 30 at -200C and day 30 at -410C it indicated the variation of ≤ 3% change. In addition, AMY showed a <4% change up to 30 days in all 4 types of storage temperature. AM-P had a ≤ 4% change on day 5 at 170C and day 30 at 2-80C, while on day 30 at -200C and -410C it has been changed only <1.5%. Moreover, a change of LDH was optimal on 5 at 170C, while it was changed to <3% on day 30 at -200C and -410C. CK-T was stable for 25 days at 2-80C with a < 2.5% change, whereas it was stable for 10 days at -410C. Furthermore, HDL-c, TG, BIL-D, BIL-T, and PHO stayed 30 days at -410C and -200C with a lower bias rate when compared to their maximum imprecision rate.

The overall novel findings: the following tests were stable at 170C for 25 days with the maximum change of urea <2%, glucose <3%, protein total <1% and amylase 0.0%.

This study tried to evaluate the stability of serum biochemicals at four different storage temperatures concurrently. Each serum biochemical has inimitable stability characteristics in diverse storage temperatures. Therefore, clinical laboratories should store specimens using appropriate temperatures as soon as possible by considering the length of storage time.

Further, the author suggests, a need for further study to determine the effects of different grade temperatures and long-time storage on routine serum analytes considering sex and age of the study individuals for the proper management of analytes stability during the storage process.

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