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THE STABILITY OF CREATININE, UREA, AND URIC ACID IN SAMPLES STORED AT DIFFERENT PREDEFINED STORAGE CONDITION

MARIAM ABBAS IBRAHIM[1], TARIG KARAR[2, 3*], EMTINAN ELAMEN[4], AYAT SALAH[4], DOAA ADEL ADAM[4], MONA ISSAM[4], MOHAMMED KARRAR[1], ELYASA ELFAKI[5], LAMA ALHAWAS[2, 3], MOHAMMED ABDULFATTAH[6]

1: Clinical Chemistry Department, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan

2: Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

3: King Abdulla International Medical Research Centre, Riyadh, Saudi Arabia.

4: Clinical Chemistry Department, College of Medical Laboratory Science, Sudan International University, Khartoum, Sudan

5: Clinical Laboratory Sciences Department, College of Applied Medical Sciences, Jouf University, Qurayyat,77423

6: Department of Health and Medical Sciences, Khawarizmi International College, Abu Dhabi UAE

***Corresponding Author: Tarig Karar: E Mail: tarig.karar@yahoo.com**

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ABSTRACT

Background:

In medical labs, storage of whole blood and other blood products such as serum or plasma is often necessary due to technical challenges or to reserve samples for future reasons such as research. The goal of this research was to look into and establish the stability of specific biochemical analytes, particularly creatinine, urea, and uric acid, under various storage time and temperature settings. Materials and procedures: Following storage, a total of three biochemical analytes in the serum of ten people were

analyzed. Following the baseline measurements, each individual's serum was aliquoted and kept at 4°C for 4, 8, and 12 hours, as well as at -20°C for 7, 15, and 30 days, before being evaluated for stability. The findings were compared to the results of the original study, which were taken from fresh samples. Results: Serum uric acid levels become statistically insignificantly differing underneath all situations at the same time as serum urea and creatinine had been demonstrating instability following extended storage; tiers considerably decreased at 4 ^{o}C in eight,12 hrs and -20°C in 7, 15, and 30 days ($P < 0.05$). In conclusion: Urea and creatinine levels in serum were affected by different storage conditions while uric acid serum levels remained stable.

Keywords: Urea, creatinine, uric acid, storage, temperature, baseline measurement

INTRODUCTION:

Creatinine and urea are nonprotein nitrogenous c ompounds that are eliminated from the body through the kidney, as well as uric acid which is the end product of purine metabolism in humans, therefore the levels of these three substances in human blood were used as valuable laboratory tests to evaluate renal function and to assess the progression of renal insufficiency.

Clinical laboratory assessments are utilized by clinicians to diagnose, comply with up and analysis in sufferers with various ailments. The exactness of test findings is influenced by a variety of factors, primarily preanalytical, analytical, and normal biological types. Sample collection and handling, nutrition, exercise, and medicines are all examples of preanalytical variables that might influence test findings. Bias and imprecision are two important aspects of every test. Imprecision, often known as a lack of repeatability, is caused by both physiological and analytical variables. [1, 2]

In the whole area of clinical laboratory, including clinical biochemistry tests, the pre-analytical stage is the most significant portion of the complete analytical stages and procedure, which has an impact on patient reports.[3] It is conceivable to reanalyze stored samples to validate earlier findings or do further analysis; however, the analytes' stability should be ensured before dispatching results or initiating new investigations. Furthermore, assessing incorrect samples often results in excessive costs and the lengthening of the whole testing procedure. [4]

Maintaining the consistency of serum analytes during sample preservation is a constant challenge in clinical labs. For short periods, samples are stored at 4-8° C, while for longer periods, they are held at -20° C.[5] Some laboratories face a range of obstacles, such as equipment failure and a shortage of reagents, which may make same-day sample processing

impossible. As a result, the sole option is to store the samples at -20 C. Furthermore, samples are sometimes kept for a long period before being utilized for the study.[6] The influence of storage conditions on the stability of different serum components has been studied in the past.[7,8] Furthermore, the majority of these investigations used animal material.[9] However, little is known regarding the stability of commonly used clinical biochemical analytes in human blood, particularly the effects of keeping serum at temperatures as low as -20 ^{0}C. Serum and other blood products must often be stored in labs due to technical concerns or to preserve samples for future study. As a consequence, the temperature at which the samples are held, as well as the length of time they are kept at that temperature, are crucial pre-analytical variables that might influence analysis outcomes in the clinical biochemistry laboratory. As a result, the present research will look at the stability of three common biochemical analytes (urea, creatinine, and uric acid) in freshly isolated blood after storage at a certain temperature for various lengths of time.

MATERIALS AND METHODS:

Study design, area and population: Ten random samples from patients visiting outpatient clinics in Sudan's Khartoum state were used in this sanatorium-based study. Each patient's blood samples were the most effective for health practitioner-ordered laboratory testing, and no more blood was supplied to them.

Ethical approval: The scientific committee of the Clinical Chemistry Department, College of Medical Laboratory Sciences of Sudan International University approved the research proposal. After receiving approval from the institution, all donors gave their informed consent.

Sample collection and analysis: 10 ml of venous blood was collected from 10 individuals (after obtaining their informed consent) under aseptic conditions, the samples were allowed to form clots before centrifugation at 3000 rpm then the serum was obtained and inspected for lipemia, hemolysis or jaundice to avoid the possibility of interference. Following the baseline measurements, each man's or woman's serum was aliquoted and held at 4° C for 4, 8, or 12 hours. They were stored at -20° C for 7, 15, and 30 days before being tested for stability. The analysis of creatinine, urea, and uric acid was performed in the same outpatient clinic mentioned above on Mindary -300 auto analyzer. The results of measures taken at different time intervals were compared to baseline values.

Data management and analysis: The findings are statistically compared to the preliminary analytical measures obtained from dazzling samples using the ANOVA test. P-value becomes taken into consideration substantially when less than 0.05. To ensure the accuracy and

validity of the results, pathological and regular manage sera were examined for creatinine, urea, and uric acid.

RESULTS:

Figure 1-6 show that the mean and std deviation for all parameters preserved at 4 ^{o}C and -20 ^{o}C respectively, while table 3 and 4 show mean and std deviation for all parameters analyzed at different times and preserved at 4 ^{o}C and -20 ^{o}C respectively.

Table 5 show the differences between baseline measurement as control for all parameters and measurement after hours storage at 4 ^{o}C, while table 6 show differences between baseline measurement for all parameters and measurement after week/s storage at -20 ^{o}C

The results of our study revealed that; all measurements of serum uric acid level were stable at all condition of storage and in different times interval, while some measurements of serum urea and creatinine levels showed statistical significant decrease (table 5 & 6).

Figure1

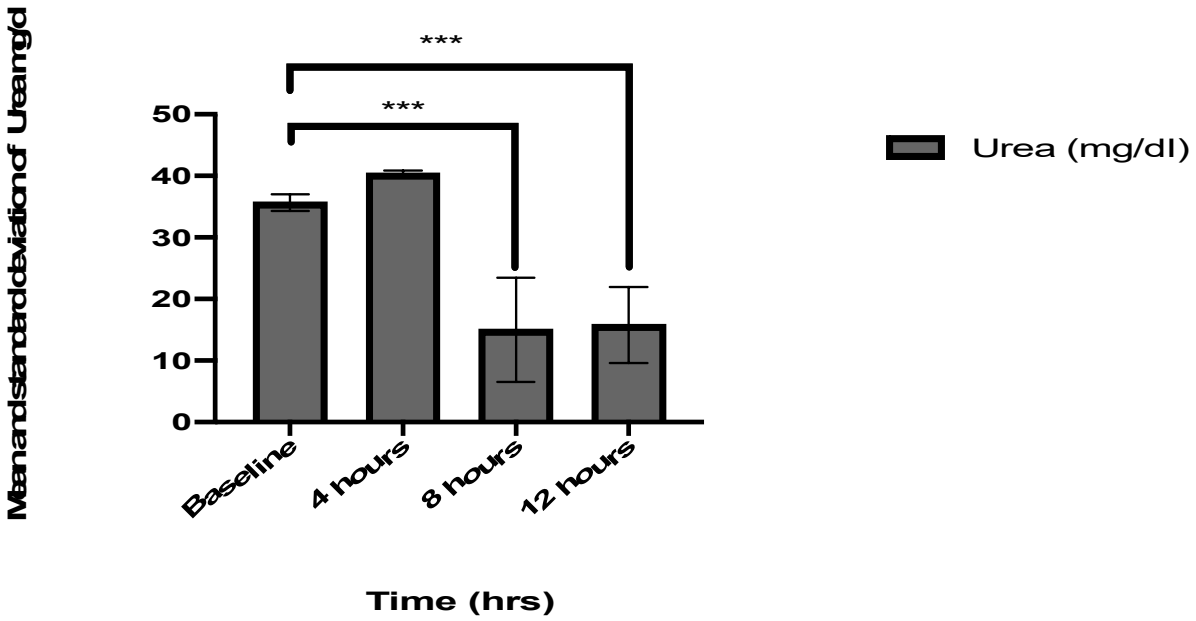


Figure 2

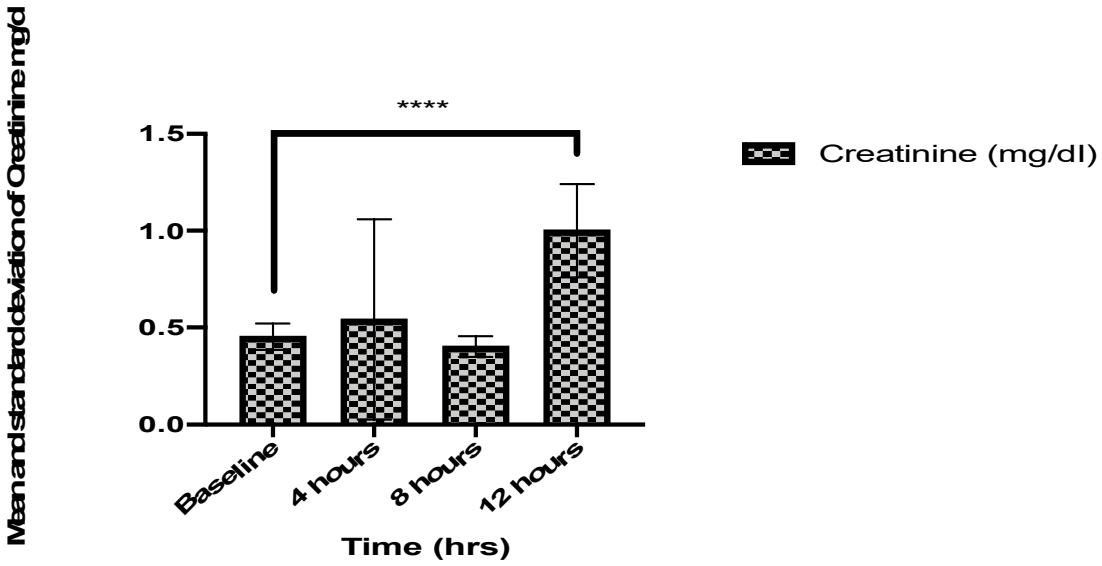


Figure 3

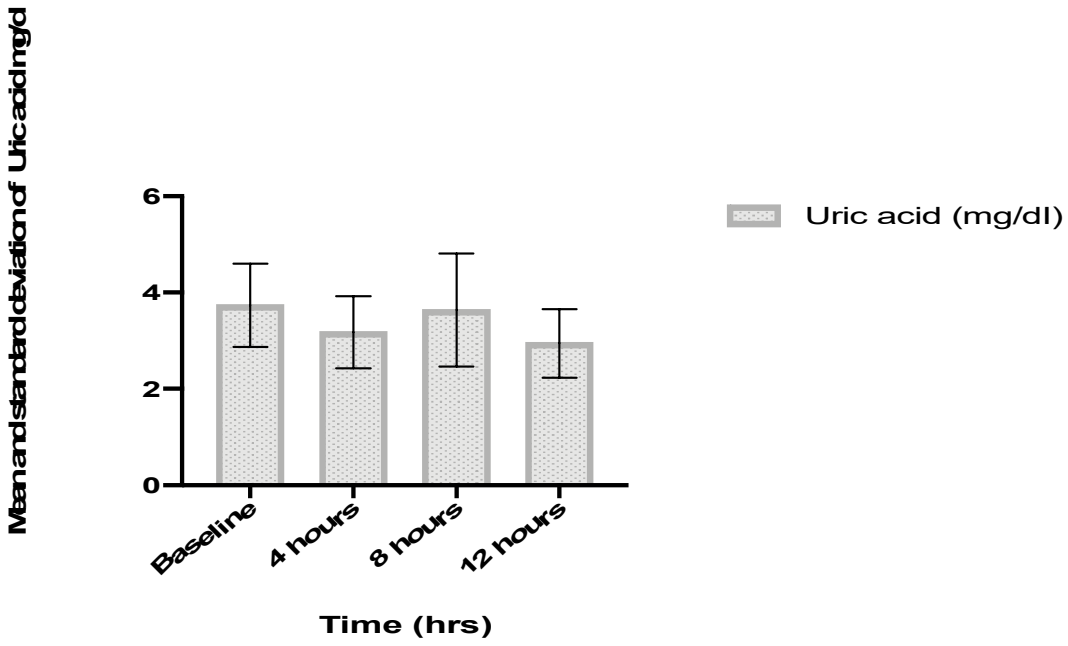


Figure (1, 2, 3): Show mean and standard deviation of urea, creatinine, and uric acid stored at 4 °C and analyzed at different times

Figure 4

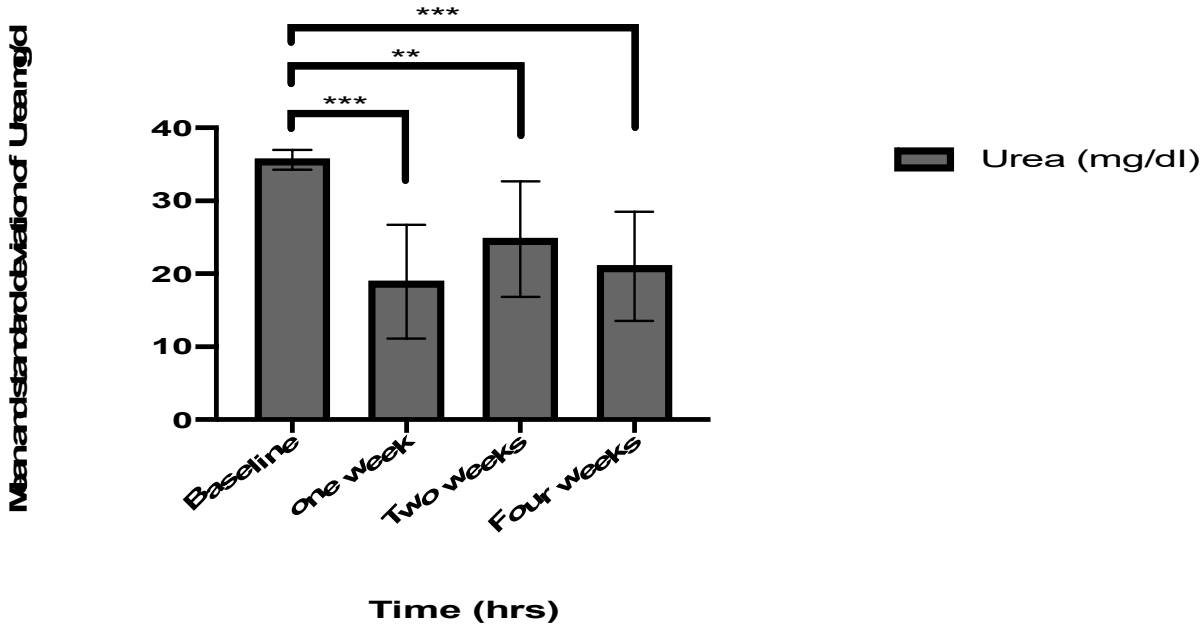


Figure 5

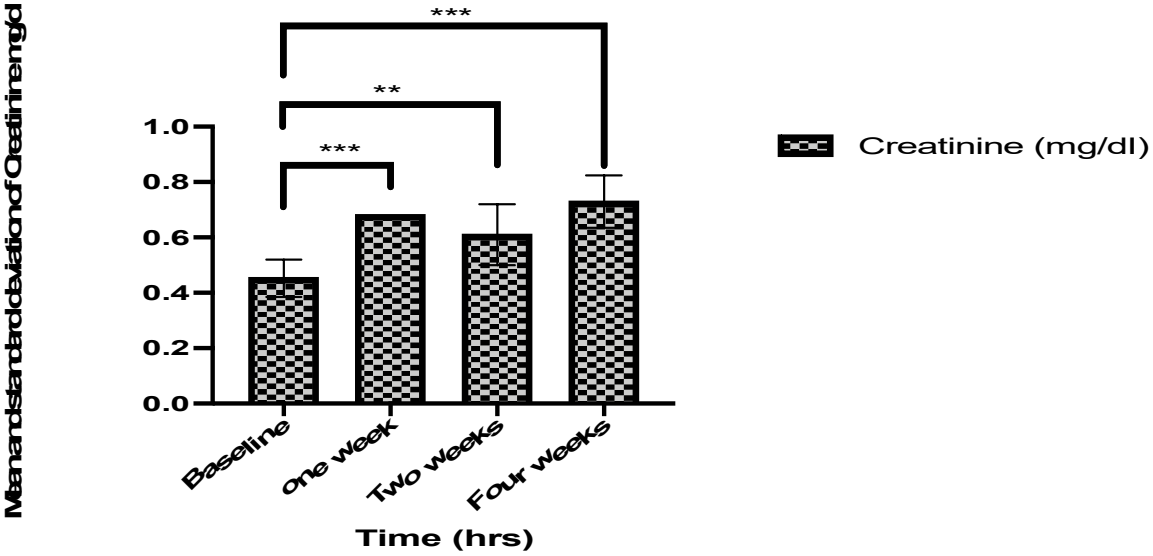
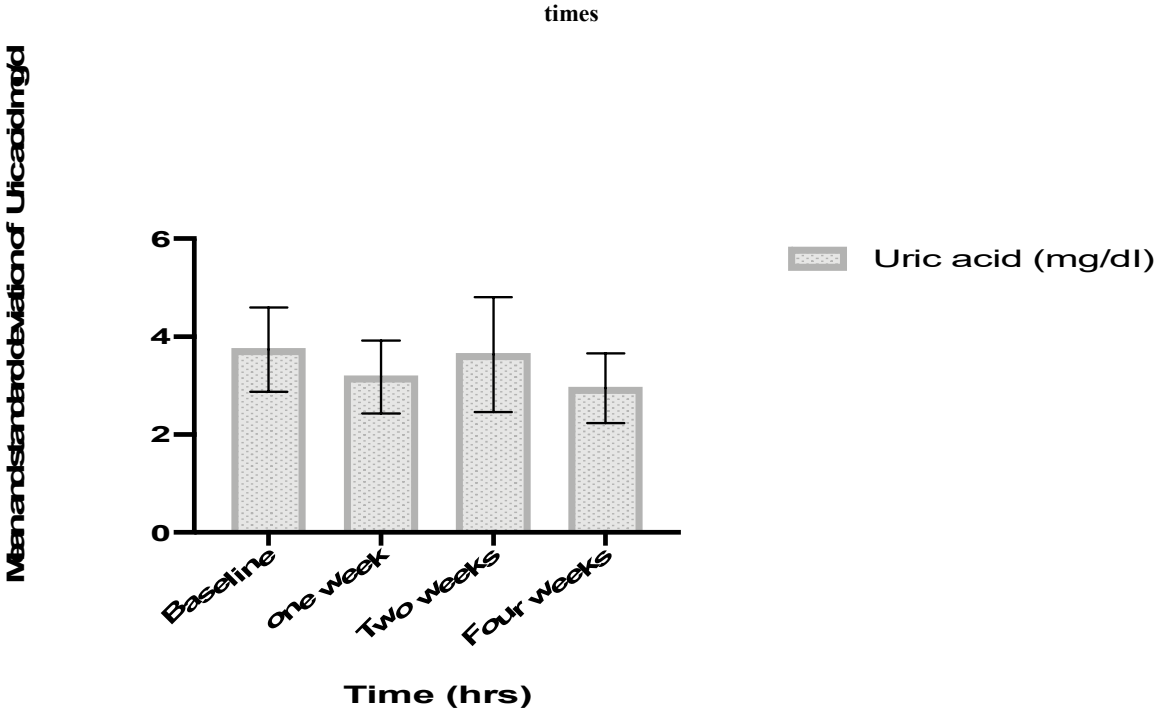


Figure 6



Figures (4 ,5, 6): Show mean and standard deviation of urea, creatinine, and uric acid stored at -20 ^{o}C and analyzed at different

Table 3: Mean and standard deviation of urea, creatinine and uric acid stored at 4 ^{o}C and analyzed at different times

Hours		Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Baseline	Mean	35.6300	0.4530	3.7200
	Std. Deviation	1.34627	0.06767	0.86255
4 Hours	Mean	40.3000	0.5410	3.9500
	Std. Deviation	9.85179	0.51692	1.40811
8 Hours	Mean	14.9900	0.4020	3.6950
	Std. Deviation	8.46108	0.05412	0.87320
12 Hours	Mean	15.7400	1.0000	3.6400
	Std. Deviation	6.19699	0.24037	0.79470
Total	Mean	26.6650	0.5990	3.7512
	Std. Deviation	1.49479E1	0.36639	0.98251

Table 4: Mean and standard deviation of urea, creatinine and uric acid stored at -20 ^{o}C and analyzed at different times

		Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Baseline	Mean	35.6300	0.4530	3.7200
	Std. Deviation	1.34627	0.06767	0.86255
One week	Mean	18.8900	0.6800	3.1700
	Std. Deviation	7.78681	0.19322	0.74692
Two weeks	Mean	24.7400	0.6100	3.6300
	Std. Deviation	7.92523	0.11005	1.17289
Four weeks	Mean	21.0000	0.7300	2.9400
	Std. Deviation	7.49815	0.09487	0.71212
Total	Mean	25.0650	0.6182	3.3650
	Std. Deviation	1.12227	0.16045	0.91723

Table 5: Comparison between baseline measurement and measurement after hours storage in 4 °C

Variables	Hours	Control	P. value
Urea (mg/dl)	4 hrs	Baseline	0.588
	8 hrs	Baseline	0.000
	12 hrs	Baseline	0.000
Creatinine (mg/dl)	4 hrs	Baseline	0.835
	8 hrs	Baseline	0.960
	12 hrs	Baseline	0.000
Uric acid (mg/dl)	4 hrs	Baseline	0.922
	8 hrs	Baseline	1.000
	12 hrs	Baseline	0.996

Table 6: Comparison between baseline measurement and measurement after week/s storage at -20 °C

Variables	Weeks	Control	P. value
Urea (mg/dl)	One week	Baseline	0.001
	Two weeks	Baseline	0.039
	Four weeks	Baseline	0.004
Creatinine (mg/dl)	One week	Baseline	0.001
	Two weeks	Baseline	0.022
	Four weeks	Baseline	0.000
Uric acid (mg/dl)	One week	Baseline	0.386
	Two weeks	Baseline	0.992
	Four weeks	Baseline	0.143

DISCUSSION:

Our outcomes have a look at indicating that almost all of the tested metabolites were solid, and no clinically extensive variations have been determined for analytes tiers. Following one-of-a-kind storage periods in comparison to the baseline samples measurements; however, some of our serum findings are not constant with the findings of studies. These conflicting results may be because the stability of serum analytes throughout the garage of serum samples is often incomplete and, once in a while, contradictory.

Urea; Increase when storage for short duration [4hrs] at 4°C, after that showed significant decrease, when stored for long duration [week/ 2 weeks/ month] at -20°C also showed significant decrease from the first week.

Consistent with a previous study [5]. We did now not stumble on any statistically or clinically tremendous trade in urea ranges. Moreover, urea ranges found a boom inside the values over a brief time from baseline size time. However, urea instability, indicated by way of an enormous lower in fields, particularly at a long time after baseline size in unique temperatures., These findings believe the ones of a preceding look at reporting that serum urea degrees exhibited a boom inside the values and a massive decrease (15.6% on average) in levels 10, 11

Cratinine; When stored for a short duration [4/8/12hrs] at 4°C confirmed exchange after 8hrs, for long-length [week/ 2 weeks/ month] at -20°C showed a significant lower after weeks.

Verenker *et al*. Their examination found the impact of storage and temperature on biochemical analytes, creatinine, and uric acid, in pooled serum samples saved at -20c. Mean creatinine concentration in all ten pooled serum samples become located to boom above the

everyday tiers after storage for ten days at -20°C; urea attention at -20°C changed into observed to grow handiest in two samples. However, the opposite eight samples were strong and showed outcomes beneath the regular variety [12]

Previous study [13] reported that the concentration of phosphorus and creatinine in serum increased with increasing temperature when compared with base line results, this changes in creatinine levels could be due to proper storage temperature and time or may be due to technical or instrumental adjustment, accordingly the result of present study showed significant increase in creatinine levels between base line measurement and stored sample for weeks. Moreover, the results of this study were not in agreement with the results of other previous study [14].

Uric acid size within the present look confirmed not many time-based exchanges relative to the baseline size or trend through the years after up to one month of a garage at exclusive temperatures cited before. This result is constant with a preceding look at [15, 16]. Furthermore, no static differences were discovered among some biochemical metabolites, including uric acid, while measured in a new pattern and inside the sample saved at -20 °C for 7,15, and 30 days.

CONCLUSION:

While all the three parameters showed no clinically significant differences, urea and creatinine showed statistically significant change and affected by storage condition, while uric acid showed stability in serum levels relative to baseline or trend over time after up to four weeks.

Recommendations:

If a extent isn't always to take region right now after specimen collection, proper garage temperatures and times have to be considered for analytes going for subsequent storage. Moreover, recording the duration of time from series to separation of each pattern would possibly allow suitable adjustments for the benign growth in the awareness of these analytes over the years.

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