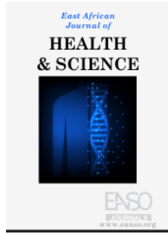

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Original Article

Stability of Selected Biochemical Analytes in Serum Stored at Room Temperature for 24 Hours at Kericho County Referral Hospital, Kenya

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Biochemical, Analytes, Stability, Room Temperature, Reference Change Value, Mean Percentage Difference.

ABSTRACT

Accurate and reliable medical laboratory test results are essential for correct patient diagnosis and management. This is however influenced by many preanalytical, analytical, and post-analytical factors. A general problem faced by most clinical laboratories is the inability to timely process all blood samples received due to overwhelming workload, reagents stock-outs, and equipment breakdown, necessitating storage which predisposes analytes to deterioration. To ensure accurate results from stored samples, the determination of the stability of the analytes under various environmental conditions and storage periods is important in informing acceptable sample handling and storage. This study was designed to experimentally observe changes and evaluate the stability of eleven biochemical analytes in serum samples from 20 randomly sampled healthy volunteers stored at room temperature over 24 hours at Kericho County Referral Hospital laboratory. 10mls of venous blood was drawn from each volunteer, serum was separated and five aliquot sets were prepared. Analysis was done on a set immediately, at 2, 4, 8, and 24 hours, to determine the concentration of the selected analytes on Human Star 100 chemistry analyser. Student pair T-test and Wilcoxon rank were used to analyse the data. Chloride, Creatinine, Glucose, Potassium, Sodium, Urea, Alkaline Phosphatase, Alanine Amino Transferase and Aspartate Amino Transferase registered stability up to 24 hours, with mean percentage differences less the respective calculated reference change value of 4.54, 35.97, 13.14, 10.89, 2.16, 32.15, 16.48, 42.25 and 42.52. Direct and total bilirubin showed stability only up to 2 and 4 hours, respectively, with mean percentage differences of -2.6 and 1.07 against respective calculated reference change values of 1.14 and 0.675 in the subsequent analysis. This study recommends immediate analysis of serum

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for total and direct bilirubin, while electrolytes, enzymatic analytes and other metabolic waste and substrates can be analysed within 24 hours without extra storage precautions. If testing is delayed, then effective storage including a dark room, refrigeration, or freezing, should be applied to maintain analytes stability for reliable results.

APA CITATION

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INTRODUCTION

Accurate and timely laboratory test results, which can be obtained through careful monitoring of the testing process, are fundamental for correct diagnosis, monitoring, and prognosis in patients with different diseases (Emre *et al*., 2021). The laboratory testing process is divided into preanalytical, analytical, and post-analytical phases, and a mishap at any stage in this process will most likely end with unreliable results (Bonini *et al*., 2002, Alavi *et al.,* 2020). The testing personnel, the testing environment, the supplies, the procedure, the sensitivity and the specificity of the testing technique, and the accuracy and the precision of the equipment are all factors which have a bearing on results (Mrazek *et al*., 2020). Careful attention must therefore be given to every step from sample collection, handling, transport, storage, and analysis if the results are to be of the desired clinical value (Fisher *et al*., 2018).

70% of all laboratory errors however have been shown to occur at the preanalytical phase, confirming increased vulnerability of this phase activities to faults (Asmelash *et al*., 2020). These include specimen collection, transportation, handling and storage; processes that can be monitored to minimise analyte variations to acceptable levels, maximising the reliability of laboratory test results for diagnosing diseases (Najat, 2017). Though normalised by many laboratories, these processes may cause significant changes in biochemical analytes, causing incorrect results that may lead to wrong diagnosis and poor patient management (Flores *et al.,* 2020).

Serum, which is obtained from clotted blood by centrifugation, is a commonly used specimen for analysis in the clinical chemistry laboratory (Carey *et al*., 2016). The analytes in the serum are susceptible to changes and must be carefully harvested, handled, stored, and analysed (Kachhawa *et al.,* 2017). Analysis using freshly drawn serum or plasma is highly recommended to guarantee acceptable results because many environmental factors can affect the viability of the sample and the stability of the analytes (Flores *et al.,* 2020). The interval between sample collection and processing

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should be minimised to reduce changes in analytes activities and concentrations (CLSI, 2010; Ikeda *et al.,* 2020). Delays in the testing process or reuse of the samples for missing results or added tests are sometimes inevitable in routine practice; hence the stability of analytes is a major concern in clinical chemistry (Zemlin, 2018).

Interrupted power supply, lack of reagents, equipment malfunction, unprecedented workload, and late sample reception are some of the factors that frequently necessitate the storage of specimens in clinical laboratories (Damtie *et al.,* 2020). Other factors may include research work which calls for later analysis, batch testing, additional test requests, and all requiring sample analysis hours to days after collection (Camargo *et al.,* 2020). Also, the evolving scenario of consolidation of smaller laboratories to create a core laboratory, which has become popular the world over, creates extra-pre-analytical factors on the patients' samples, including different transportation, storage, and handling conditions that may interfere with the stability of analytes (Magnette *et al*., 2016).

Duration of specimen storage has been noted to greatly influence prescription drug concentration, analyte values, and enzyme stability in the blood specimen. For example, Damtie *et al*. (2020) found that variable temperatures and lengths of storage duration changed the stability of Nevirapine, a highly prescribed drug in the treatment and management of HIV patients. Serum or plasma stored at 25 ºC had significant changes in nine of the blood profile tests included in the comprehensive metabolic panel, where glucose levels were significantly different after being stored for four hours. Potassium, creatinine, and total protein results were significantly different when tested at thirty hours; calcium at forty hours; albumin and alanine aminotransferase at forty-eight hours; and CO_2 at fifty-six hours, while urea concentration remained variable through the storage period (Kachhawa *et al*., 2017; Razi e*t al*., 2020).

The various analytes have stability limits, which is the point in time when the percentage deviation (PD %) of the analyte reaches the maximum allowable error (Emre *et al.,* 2021). Stability limits should be defined for each analyte, and these can be used as a rejection for samples before processing (Gómez-Rioja *et al.,* 2019). A number of experimental studies have assessed the stability of most laboratory analytes under various conditions, with different and sometimes inconsistent results due to the lack of standard experimental designs and wide variability in maximum permissible instability (MPI) specifications (Emre *et al.,* 2021). Also, there are few clinical guidelines with general recommendations for the laboratories (Shimizu & Ichihara, 2019). Very few of these studies have been done in Kenya, particularly in Kericho County Referral Hospital KCRH and its environs despite the geographical uniqueness (Beale *et al.,* 2016).

The hospital's large capacity attracts many patients and specimens leading to a high workload in the laboratory, coupled with the developing world challenges including strained resources, prompts delayed specimen analysis, making it necessary to evaluate the stability of these analytes for evidence-based local recommendations and guide for reliability of results when such delays occur, was the primary purpose of this study.

Study Objectives

The main objective of this study was to determine the stability of the selected biochemical analytes in serum left at room temperature, pressure, and humidity for 24 hours at the Kericho County Referral Hospital laboratory. The specific objectives were:

- To determine the over-time concentration variations of selected biochemical analytes in serum samples stored at room temperature at the Kericho County Referral Hospital Laboratory.

- To determine the stability of selected biochemical analytes in serum samples stored at room temperature on the laboratory working bench over 24 hours at the Kericho County Referral Hospital Laboratory.

Limitation of the study

The study only sampled a few of the biochemical analytes due to financial constraints limiting the generalizability of the findings to other serum biochemical analytes.

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Delimitation of the study

The study sampled analytes from all the main categories of biochemical analytes broadening the scope of generalisation.

MATERIALS AND METHOD

Study design

The study utilised experimental design and real-time analysis of serum specimens for the selected analytes at the specified time intervals.

Study Site

The study was conducted at Kericho County Referral Hospital laboratory in Kenya, which is the largest referral hospital laboratory in the county. Kericho County is located in the highlands of Kenya, at altitude levels of 4500 feet (1500 m) and 6750 feet (2250 m) above sea level. The county receives rainfall ranging from 1200 mm and 2500 mm annually (the rainfall pattern is unimodal), while the temperature ranges between 12 °C and 28 °C.

Target Population

The target population was healthy volunteer adults living within Kericho County.

Exclusion Criteria

Adults on medication, children, and pregnant women were excluded from the study. Haemolyzed, icteric, and lipemic serum samples were also excluded from the analysis.

Sampling Technique

The study used simple random sampling to select the volunteers who consented. A single venepuncture was performed on each volunteer. Routinely investigated clinical chemistry analytes in the enzymatic, electrolytes, metabolic substrates, and waste molecules were purposively selected. Commonly requested analytes from each category were also picked purposively to represent the entire group.

Sample size

From Yamane's formula, a total of 99.25 aliquots were required for this study. Five aliquots from each volunteer were used to calculate the number of participants that were recruited.

$$n = \frac{N}{1+N(e)^{2}} \qquad \text{(i)}$$

Yamane's 1967 sample size formula. Where: n:Sample size (calculated from the population); Population (132) –Average no of clinical chemistry profiles ordered daily at the Kericho County Referral hospital laboratory); e: The level precision (0.05)

n = N/ (1+N $(0.05)^2$)=99.25 (no of aliquots needed)

99.25/5 (aliquots per volunteer) =20 participants

Sample Collection, Processing, Storage, and Analysis

Vacuette® closed collection system was used to obtain 10mls of venous blood from each of the twenty volunteers. After 30 minutes of standing, the samples were centrifuged at 3000 revolutions per minute for five minutes. Serum specimens were examined for haemolysis and lipemia to prevent possible analysis interference from impacting the results. 400 µl of serum specimen from each subject was aliquoted into five 1 mL vials, which were kept on the working bench. One aliquot was analysed at baseline, then the other vial sets at 2, 4, 8, and 24 hours sequentially, using the same methodology and under the same conditions, on the Human Star 100 chemistry analyser. The equipment was qualified by calibration and independently checked using commercial quality control materials before specimen analysis.

Data was cleaned, coded, and analysed on SPSS version 22. Summary statistics included mean, median level, and standard deviation. Normality test was done using Shapiro Wilk test and for data which was normal student t-test was applied.

To assess the statistical significance of the difference between serum aliquot analysed at baseline and the subsequent results, student pair t-

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test and Wilcoxon rank were used. The difference between means and median was calculated using the following formula:

$$\frac{Tx-T0}{T0}*100$$

Where: $T0$: Initial mean or median value; Tx: Mean or median of the measured values

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

RCV was calculated using the formula:

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

Where: CVa - Analytical variation; CVi - Biological variation; Z - is the number of standard deviations for a given probability.

When the mean or median percentage difference of an analyte is less than the RCV calculated, one infers that there are no errors in the last quantification; whereas if the mean/median percentage difference is bigger than the RCV calculated, this mirrors the potential clinical impact (Aarsand *et al.,* 2018), which referred to the instability of the evaluated analyte in this study.

RESULTS

Demographic Data of the Study Participants

A total of 20 volunteers were recruited for the study. Out of 20 participants, 14 (70%) were male, while 6(30%) were female. The age of the study participants was grouped with ages between 18-27 years old representing the largest population in the study, 9(45%), while the study participants in the 28-37 years bracket were 8(40%), and 38-47 years were 3(15%), representing the smallest population. Physical measurements of the study participants were also taken, with a majority weighing 55-64 kilograms, 8(40%), while the least proportion weighed 84 kilograms and above, 1(5%), as presented in *Table 1*.

Table 1: Demographic characteristics of the study participants

Characteristic	Category	n (%)
Gender	Male	14 (70)
	Female	6 (30)
Age (Yrs)	18-27	9 (45)
	28-37	8(40)
	38-47	3 (15)
Weight (Kgs)	45-54	6(30)
	55-64	8 (40)
	65-74	3 (15)
	75-84	2(10)
	84+	1 (5)

Data presented are the number (n) and proportions (%) of participant's variables (gender, age (years) and weight (kilograms)).

Concentration Changes and the Stability of the Selected Biochemical Analytes on Stored Serum Samples

The stability of stored serum samples for selected biochemical analytes was evaluated following analysis at 2, 4, 8, and 24 hours and the results are presented in *Table 2*. Variations were noted in the results of all the analytes during the 24 hours period. A comparison between the analytes 'mean percentage difference and reference change value (RCV), however, revealed that some analytes were generally more stable and could resist environmental effects over longer periods compared to some that showed very rapid changes denoting instability on exposure to uncontrolled environmental conditions.

From the enzymatic analytes category, all the selected enzymes showed stability up to 24 hours post extraction. Alkaline phosphatase (ALP) registered a lower mean percentage difference of

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0.50, 0.86, 2.08, and 1.17% at 2, 4, 8, and 24 hours respectively, compared to the calculated RCV of ±16.48, which was evidence of good stability. Similar observations for stability were made with alanine aminotransferase (ALT), which registered mean percentage differences of -1.67, -4.05, -3.76, and -8.27%, all of which were less than the calculated RCV of ±42.25. Also, aspartate aminotransferase (AST), another enzymatic analyte, showed good stability with mean percentage difference values of 2.30, 1.71, 3.04, and -0.48%, all of which were less than the calculated RCV of ±42.51.

Analysis of electrolytes sodium, potassium, and chloride revealed relative stability within the 24 hours period. Chloride analyte depicted stability all through 2, 4, 8, and 24 hours registering mean percentage differences of 1.04, 0.98, 1.69, and -0.36%, which were less than the calculated RCV of ±4.54 and revealing no potential clinical impact. Likewise, potassium showed evidence of stability, scoring means percentage differences of 0.47, 0.70, 0.70, and 2.11%, all of which were less than the calculated RCV of ±10.89 hence no potential clinical impact on results. Similarly, the sodium analyte depicted stability at room temperature over the 2-, 4-, 8-, and 24-hour analysis period, scoring mean percentage difference of 0.68, 0.72, 1.30, and 0.60%, respectively, in comparison to the calculated RCV of ±2.16 hence no potential clinical impact on the results.

The substrates category and the waste molecules categories also registered a high degree of stability when analysed within a day. Glucose's percentage mean difference scores were 0.43, 0.43, 0.65, and -5.90%, which were less than the calculated RCV ± 13.14, signifying no potential clinical impact over the period. Urea analyte also showed stability when serum samples were left for 2, 4, 8 and 24 hours at room temperature and analysis was done as their mean percentage difference of 0, 0.91, 0, and -2.13% were less than the calculated RCV of ±32.15. Creatinine equally showed stability registering mean percentage differences of 1.01, 0.14, 1.08, and -3.0%, all less than the calculated RCV of ±35.97.

There was however evidence of instability with bilirubin which registered diminishing scores with significant variations over the 24 hours period. Direct bilirubin analysed at 4, 8, and 24 hours when serum was left at room temperature registered mean percentage differences of -2.6, -5.1, and -10.3%, which were greater than RCV of ±1.14, showing significant deterioration and potential clinical impact of the results. This analyte was only stable when the analysis was done within 2 hours, at which the mean percentage difference was zero, a value less than the RCV. Similarly, total bilirubin showed evidence of instability when serum samples were analysed at 8 and 24 hours at room temperature, where the mean percentage difference was 1.07 and -2.62%, respectively, all greater than RCV of ±0.675. This analyte however was observed to remain stable with no significant deterioration and potential clinical impact when serum samples were left on the bench for up to 4 hours, scoring a percentage mean difference of 0.6 and -0.48% at the 2nd and 4th-hour analyses, which were less than the RCV.

Table 2: Results of the effect of storage time on the concentration and the stability of the selected biochemical analytes on stored serum samples

Analyte (Units)	Time(hrs)	Mean (SD)	Mean %difference	P value	RCV	Clinical Impact
Direct Bilirubin (µmol/L)	Baseline	3.9(1.107)	-	-	1.14	No
	2	3.9(1.082)	0	0.915		
	4	3.8(1.046)	-2.6	0.014		Yes
	8	3.7(1.058)	-5.1	0.010		
	24	3.5(1.050)	-10.3	0.000		
Total Bilirubin (µmol/L)	Baseline	10.31(4.497)	-	-	0.675	No
	2	10.24(4.554)	-0.68	0.617		
	4	10.26(4.203)	-0.48	0.599		Yes
	8	10.42(4.317)	1.07	0.350		

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Analyte (Units)	Time(hrs)	Mean (SD)	Mean %difference	P value	RCV	Clinical Impact
	24	10.04(4.313)	-2.62	0.062		
Chloride (mmol/L)	Baseline	113.02(2.829)	-	-	4.54	No
	2	113.50(2.689)	1.04	0.018		
	4	113.58(2.927)	0.98	0.017		
	8	112.88(2.642)	1.69	0.564		
	24	112.88(2.765)	-0.36	0.497		
Creatinine (µmol/L)	Baseline	69.45(11.34)	-	-	35.97	No
	2	69.20(10.851)	1.01	0.555		
	4	69.25(11.364)	0.14	0.629		
	8	69.95(10.928)	1.08	0.116		
	24	68.05(11.213)	-3.0	0.017		
Glucose (mmol/L)	Baseline	4.60(0.548)	-	-	13.14	No
	2	4.62(0.548)	0.43	0.109		
	4	4.62(0.541)	0.43	0.057		
	8	4.61(0.540)	0.25	0.496		
	24	4.33(0.539)	-5.9	0.000		
	Baseline	4.27(0.297)	-	-	10.89	No
Potassium (mmol/L)	2	4.29(0.297)	0.47	0.000		
	4	4.30(0.293)	0.70	0.001		
	8	4.30(0.288)	0.70	0.004		
	24	4.26(0.286)	-0.23	0.802		
Sodium (mmol/L)	Baseline	138.30(2.408)	-	-	2.16	No
	2	139.25(2.531)	0.68	0.002		
	4	139.30 2.921)	0.72	0.002		
	8	140.10(3.059)	1.30	0.000		
	24	139.15(3.787)	0.61	0.80		
Urea (mmol/L)	Baseline	3.29 (0.851)		-	32.15	No
	2	3.29 (0.851)	0	1.000		
	4	3.26 (0.834)	0.91	0.030		
	8	3.29 (0.822)	0	0.716		
	24	3.22 (0.786)	-2.13	0.079		
Alkaline phosphate (U/L)	Baseline	98.6 (31.1)	-	-	16.48	No
	2	99.1(31.2)	0.50	0.005		
	4	99.4 (31.6)	0.86	0.025		
	8	100.00 (31.2)	2.08	0.000		
	24	100.1(32.1)	1.17	0.008		
Alanine aminotransferase (U/L)	Baseline	23.94 (13.0)	-	-	42.25	No
	2	23.54(13.1)	-1.67	0.183		
	4	22.96 (12.9)	-4.05	0.004		
	8	22.91 (12.84)	-3.76	0.008		
	24	21.85 (12.83)	-8.27	0.000		
Aspartate amino transferase (U/L)	Baseline	26.91 (8.71)	-	-	42.51	No
	2	27.53(8.716)	2.30	0.001		
	4	27.39 (8.75)	1.71	0.013		
	8	27.83 (8.67)	3.04	0.001		
	24	27.03 (8.75)	-0.48	0.407		

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Table showing the concentrations, the MPD versus RCV at 2, 4, 8 and 24 hours

DISCUSSION

The Effect of Time on the Concentration and the Stability of the Selected Biochemical Analytes on Stored Serum Samples

Despite the stability of most of the analytes, the findings of this study generally show overtime variation in concentrations. The changes shown by enzymes, electrolytes, and most of the substrates and waste molecules, however, were insignificant, except for bilirubin, which had marked changes, signifying potential clinical impact if analysis of sample is delayed.

The enzyme ALP showed minimal changes in this study, with reliable results obtained at every analysis within 24 hours of the sample collection. This was consistent with the findings by Dittadi *et al.* (2019) who determined a minor increase of ALP activities in serum samples from its baseline values during the first 8 hours of storage. The observed changes however were within acceptable limits, as shown by the mean percentage difference that consistently remained below the calculated RCV. ALT results as well indicated that reliable results can be obtained on serum stored at room temperature and analysed within 24 hours. This was however in contradiction to the findings by Nwosu *et al.* (2009) who determined that reliable ALT results can only be obtained when the analysis is done within 16 hours of sample collection if left at room temperature. AST also gave reliable results until the 24th-hour analysis in this study. Nwosu *et al*. (2009) however found that reliable AST values without adverse clinical impact could only be obtained when analyses were done within 10 hours from sample collection. Such differences can be accounted for by population (liver disease patients vs healthy volunteers) and environmental differences.

The three electrolytes, sodium, potassium, and chloride, showed stability all through the analysis with the mean percentage difference consistently remaining less than the respective RCV. Sodium and potassium showed no patterns of deterioration. The values from all subsequent analyses remained quite similar to the baseline value except for random acceptable deviations with repeat testing. Chloride on the other hand indicated a progressive decrease in values with time, registering a mean percentage difference of -0.36% at the 24th-hour analysis. The values did not show any substantial variation from baseline, signifying no adverse clinical impact when the analysis was done within 24 hours of sample collection. This mirrors findings from a study by Bobby *et al*. (2002) who investigated the stability of 24 analytes after prolonged contact of plasma and serum with blood cells and after immediate separation of plasma and serum at room temperature (25 ^{o}C) and analysed periodically from baseline to 56th hour. They concluded that sodium, potassium, and chloride remained stable in serum and plasma for up to 56 hours. Bobby *et al*. (2002) also recorded differences of <5% for electrolytes when serum samples were analysed after 24 hours under room temperature storage.

Glucose concentrations also remained fairly constant for the first 8 hours followed by a drastic decrease of 0.27 mmol/L by the 24th-hour analysis. Despite this rapid decrease, the results remained clinically significant with mean percentage change values way less than the RCV. This therefore shows that blood glucose can be analysed on samples left on the working bench at room temperature for up to 24 hours giving reliable results. A study by Bobby *et al*. (2002) found a cumulative 2.5 mmol/L net loss of glucose at 24 hours which is bigger than the changes registered in this study.

Similarly, reliable results were obtained for urea with no significant adverse clinical impact within 24 hours. The reading at 24 hours only differed from the baseline value by 0.07 mmol/L (2.12%), which was in agreement with the findings of a previous study by Dittadi *et al*. (2019) that reported that there would be no effect on serum samples stored at room temperatures for as long as 72 hours for urea analyte with <5% percentage difference. The same was observed with the creatinine analyte which gave reliable results with minimal variations over the 24-hour period that the samples were left at room temperature. Recorded values in this study showed a slight increase in creatinine concentration with subsequent analysis during the sample storage period. Non-specific formation of pseudo-creatinine with kinetic Jaffe reaction accounts for this

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observed increase, as explained in findings by Hedayati in their study (Hedayati et al. 2020).

Unfortunately, for direct bilirubin, values with no adverse clinical impact were only recorded at the 2nd-hour post-baseline. All the subsequent analyses registered values that showed considerable deterioration, with a mean percentage difference way above the RCV. Similarly, total bilirubin registered acceptable values only up to the 4th-hour analysis, beyond which massive deterioration was recorded. Results at the 8th and 24th hours were unreliable, with adverse clinical impact, scoring a mean percentage difference much higher than the RCV. From this, it is evident that bilirubin, both total and direct, is very unstable and rapidly decomposes when samples are left at room temperature. Results obtained beyond 2 hours for direct and 4 hours for total bilirubin could mislead and cause adverse clinical consequences if relied upon for diagnosis or monitoring of patients' management. This was contrary to findings by Sofronescu and the team who concluded that no significant effects would occur on bilirubin results with delays up to 8 hours at room temperature and without protection from light (Sofronescu *et al.*, 2012). Variations in the extent and intensity of light exposure, which is a known decomposer of bilirubin, probably explain the gap.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

From the findings of this study, all the analytes gave different readings with time, showing that results obtained several hours after sample collection are quite often lower than the baseline values (presumed true value) and relatively higher for creatinine and must be carefully interpreted in the clinical set-up. Nine of the eleven biochemical analytes investigated representing the three categories; enzymes (ALP, ALT, and AST), electrolytes (sodium, potassium, and chloride), metabolic substrates and waste molecules (glucose, creatinine, and urea) were stable when samples were left at room temperature and analysis was done within 24 hours, except for direct and total bilirubin that only registered reliable results within 2 and 4 hours respectively.

This means that biochemical analysis can be performed on samples left at room temperatures for a day without adverse clinical impact except for direct and total bilirubin, which must be evaluated immediately and not later than 2 and 4 hours respectively, otherwise; special storage conditions need to be defined for reliable and clinically valuable results.

Recommendation

This study recommends that serum samples for enzymes; alkaline phosphate, alanine aminotransferase and aspartate aminotransferase, electrolytes; sodium, potassium, and chloride, metabolic substrates, and waste materials; glucose, creatinine, and urea, should be analysed in the laboratory preferably within 24 hours of samples of collection to ensure valid and reliable results without any specialised storage. Direct and total bilirubin however should be analysed immediately, otherwise not later than 2 and 4 hours, respectively when the serum samples are left at room temperature. Specialised storage must be considered if these timelines cannot be met.

This study also recommends further studies to evaluate and compare the stability of these analytes when storage time is prolonged beyond 24 hours and under various storage conditions available at the facility and in other set-ups to establish a best practice that would ascertain the reliability of results whenever analysis cannot be performed immediately.

REFERENCES

Aarsand A. K., Røraas T., Fernandez-Calle P., Ricos C., Díaz-Garzón J., Jonker N., Perich C., González-Lao E., Carobene A, Minchinela J., Coşkun A., Simón M., Álvarez V., Bartlett W. A., Fernández-Fernández P., Boned B., Braga F., Corte Z., Aslan B., … & Sandberg S. (2018). The Biological Variation Data Critical Appraisal Checklist: A Standard for Evaluating Studies on Biological Variation. *Clin Chem*, 64:501-514

Alavi, N., Khan, S. H., Saadia, A., & Naeem, T. (2020). Challenges in preanalytical phase of laboratory medicine: rate of blood sample nonconformity in a tertiary care hospital. *EJIFCC*, *31*(1), 21.

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Article DOI: https://doi.org/10.37284/eajhs.5.1.844

Asmelash, D., Worede, A., & Teshome, M. (2020). Extra-analytical clinical laboratory errors in Africa: a systematic review and meta-analysis. *EJIFCC*, *31*(3), 208.

Beale, D. J., Jones, O. A., Karpe, A. V., Dayalan, S., Oh, D. Y., Kouremenos, K. A., ... & Palombo, E. A. (2016). A review of analytical techniques and their application in disease diagnosis in breathomics and salivaomics research. *International journal of molecular sciences*, *18*(1), 24.

Bobby, L., Boyanton, Jr., & Blick, K. (2002). Stability of twenty-four analytes in human serum. *Clinical Chemistry*, 48(12), 2242-2247.

Bonini, P., Plebani, M., Ceriotti, F., & Rubboli, F. (2002). Errors in Laboratory Medicine. *Clinical Chemistry*, 48(5), 691 - 698.

Camargo, Fabricio et al. 2020. "Serum Biochemistry of the Arrau Turtle (Podocnemis Expansa): Frozen Analyte Stability and the Effects of Long-Term Storage." *Veterinary Clinical Pathology* 49(1): 42–47.

Carey, R. N., Jani, C., Johnson, C., Pearce, J., Hui-Ng, P., & Lacson, E. (2016). Chemistry testing on plasma versus serum samples in dialysis patients: clinical and quality improvement implications. *Clinical Journal of the American Society of Nephrology*, *11*(9), 1675-1679.

Clinical and Laboratory Standards Institute (CLSI). (2010). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. Wayne PA, 2010.

Damtie, T. A., Ibrahim, A. J., & Yikna, B. B. (2020). Supply chain management performance of HIV/AIDS commodities and factors affecting it at health facilities of SNNPRS of Ethiopia; from the perspective of achieving 90-90-90 strategies. *Integrated Pharmacy Research & Practice*, *9*, 11.

Dittadi, R., Fabricio, A. S., Rainato, G., Peroni, E., Di Tonno, F., Vezzù, B., ... & Gion, M. (2019). Preanalytical stability of [-2] proPSA in whole blood stored at room temperature before separation of serum and plasma: implications to Phi determination. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *57*(4), 521-531.

Emre, H. O., Karpuzoglu, F. H., Coskun, C., Sezer, E. D., Ozturk, O. G., Ucar, F., ... & Coskun, A. (2021). Utilisation of biological variation data in the interpretation of laboratory test results–survey about clinicians' opinion and knowledge. *Biochemia medica*, *31*(1), 93-102.

Fisher, W. E., Cruz-Monserrate, Z., McElhany, A. L., Lesinski, G. B., Hart, P. A., Ghos, R., ... & Conwell, D. L. (2018). Standard operating procedures for biospecimen collection, processing, and storage: from the consortium for the study of chronic pancreatitis, diabetes, and pancreatic cancer. *Pancreas*, *47*(10), 1213.

Flores, C. F. Y., Pineda, Á. D. L. M. H., Bonilla, V. M. C., & Sáenz-Flor, K. (2020). Sample management: stability of plasma and serum on different storage conditions. *EJIFCC*, *31*(1), 46.

Gómez-Rioja, R., Amaro, M. S., Diaz-Garzón, J., Bauçà, J. M., Espartosa, D. M., & Fernández-Calle, P. (2019). A protocol for testing the stability of biochemical analytes. Technical document. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *57*(12), 1829-1836.

Hedayati, M., Razavi, S. A., Boroomand, S., & Kheradmand Kia, S. (2020). The impact of preanalytical variations on biochemical analytes stability: A systematic review. *Journal of Clinical Laboratory Analysis*, *34*(12), e23551.

Ikeda, K., Ichihara, K., Hashiguchi, T., Hidaka, Y., Kang, D., Maekawa, M., ... & Committee for Standardization, The Japanese Society of Laboratory Medicine (JSLM). (2015). Evaluation of the short-term stability of specimens for clinical laboratory testing. *Biopreservation and Biobanking*, *13*(2), 135-143.

Kachhawa, K., Kachhawa, P., Varma, M., Behera, R., Agrawal, D., & Kumar, S. (2017). Study of the stability of various biochemical analytes in samples stored at different predefined storage conditions at an accredited laboratory of India. *Journal of laboratory physicians*, *9*(01), 011-015.

 This work is licensed under a Creative Commons Attribution 4.0 International License.

Article DOI: https://doi.org/10.37284/eajhs.5.1.844

Magnette, A., Chatelain, M., Chatelain, B., Ten Cate, H., & Mullier, F. (2016). Preanalytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thrombosis journal*, *14*(1), 1-14.

Mrazek, C., Lippi, G., Keppel, M. H., Felder, T. K., Oberkofler, H., Haschke-Becher, E., & Cadamuro, J. (2020). Errors within the total laboratory testing process, from test selection to medical decision-making–A review of causes, consequences, surveillance and solutions. *Biochemia Medica*, *30*(2), 215-233.

Najat, D. (2017). Prevalence of preanalytical errors in clinical chemistry diagnostic labs in Sulaimani city of Iraqi Kurdistan. *PloS one*, *12*(1), e0170211.

Nwosu, O. K., Aloh, G. S., & Ihedioha, J. L. (2009). Changes in alt, ast and alp values of plasma and serum samples stored at refrigerator (4 0c) and room temperature (32 0c) for up to five days. *Bio-Research*, *7*(2).

Razi, F., Bandarian, F., Niazpour, F., Shafiee, G., Heshmat, R., Abdollahi, M., ... & Nasli-Esfahani, E. (2020). Stability of Thirty-Four Analytes in Blood Samples of Diabetic Patients. *Clinical Laboratory*, *66*(1).

Shimizu, Y., & Ichihara, K. (2019). Elucidation of stability profiles of common chemistry analytes in serum stored at six graded temperatures. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *57*(9), 1388-1396.

Sofronescu, A. G., Loebs, T., & Zhu, Y. (2012). Effects of temperature and light on the stability of bilirubin in plasma samples. *Clinica chimica acta; international journal of clinical chemistry*, 413(3-4), 463–466. https://doi.org/10.1016/j.cca.2011.10.036.

Yamane, T. (1967). Statistics: An Introductory Analysis, 2nd Ed., New York: Harper and Row.

Zemlin, A. E. (2018). Errors in the extra-analytical phases of clinical chemistry laboratory testing. *Indian Journal of Clinical Biochemistry*, *33*(2), 154-162.

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