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Research Article

The Effects of Storage Temperature and Time on Serum Potassium Level

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Abstract

Proper specimen storage is compulsory to avoid inaccurate laboratory results, wrong diagnosis and repeated sampling in patients. Electrolyte tests are usually performed as regular check-ups for specific medical conditions, such as decreased renal function, heart failure, and diarrhoea. Potassium is the main intracellular cation, which is important for regulating heart function and maintaining healthy nerves and muscles. This study was carried out to evaluate the effects of storage temperature and time on serum potassium levels among apparently healthy individuals. The consent of 12 participants was sought and obtained before enrolment. Chemchek Agappe potassium kits was used in ICuBio Ichaem-535 Chemistry Analyser to determine serum potassium level at different time interval and storage temperatures. The serum samples were analysed immediately after collection (control), 1 hour, 2hours, 4 hours, 24hours, 48hours, and 72hours at storage conditions of 4^{o}C and -25^{o}C simultaneously. Data were analysed using SPSS version 16. Test of probability less than 0.05($p<0.05$) was regarded as significant. There was no significant difference ($p>0.05$) in the K serum level of samples stored at 4^{o}C from 1hr to 4hrs when compared with the control. There was no significant difference ($p>0.05$) in the K serum level of samples stored at -25^{o}C from 1hr to 48hrs when compared with the control. There was a significant difference ($p<0.05$) in the serum K level of samples stored up to 24hrs and 72hrs at 4^{o}C and -25^{o}C respectively. In conclusion, storing serum samples for K determination at 4^{o}C and -25^{o}C for a maximum of 4hrs and 48 hrs respectively gives a stable, reliable and reproducible result. However, storage for up to 24hrs and 72 hours at 4^{o}C and -25^{o}C respectively gives a significantly unstable, unreliable and inaccurate result.

Keywords: Electrolytes, time, temperature, storage, accuracy

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Introduction

Laboratories play a crucial role in enabling clinicians to make a correct diagnosis, monitoring, prognosis, and follow-up of diseases in this era of evidence-based medicine (Kughapriya et al., 2019). Hence precision and accuracy of a test result are important. Fluid and electrolyte balance disorders are common in clinical practice, especially in hospitals (Amalia et al., 2021). The fluid amount and electrolyte concentration changes can cause a variety of problems if not appropriately managed. The electrolyte tests are usually performed as regular check-ups for specific medical conditions, such as decreased renal function, heart failure, and diarrhoea (Amalia et al., 2021). Potassium (K^+), Sodium (Na^+), and Chloride (Cl^-) are primary electrolytes (ions) in the body. Changes in electrolyte concentration and or ratio of anions and cations will cause changes in cell activity that can endanger life (Kusnanto, 2016). Potassium is the main intracellular cation which is important for regulating heart function and maintaining healthy nerves and muscles. Changes in plasma potassium levels (hyperkalaemia or hypokalaemia) can affect neuromuscular and heart function (Amalia et al., 2021). Clinical features of potassium disorder can be the most life-threatening disorder compared to others (Amelia et al., 2021).

A problem facing clinical laboratories is the integrity of specimens used for biochemical assays. A number of factors such as preanalytical, analytical and normal biological variations affect the accuracy of laboratory test results (Nwosu et al., 2011). Since analytical factors are being minimized by the development of new technologies, techniques, and test kits, the relative contribution of preanalytical variation becomes a more dominant element in overall test variability. Preanalytical factors such as specimen collection, handling, storage, and time of biochemical analysis all impact on test results. Wrong preanalytical results thereby leading to wrong judgment / diagnosis by the clinician (Nwosu et al., 2011). These preanalytical factors like the storage time and the storage temperature can be monitored and controlled thereby reducing the magnitude of the errors and improving the accuracy of the test results (P Kughapriya et al, 2019).

Proper specimen storage is compulsory to avoid inaccurate laboratory result, wrong diagnosis, and repeated sampling in patients. The delay in specimen analysis can be due to follow up requests from the clinicians, large sample size, shortage of qualified laboratory scientists, tool damage and unavailability of backup equipment in the laboratory (Amalia et al., 2021). Standard guidelines for blood sample handling states that, plasma or serum should be separated (20-30min) from cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analytes (Abdoljalal,2008), and this applies particularly in potassium as higher concentration is found in the erythrocytes than in the plasma. Delayed separation can lead to diffusion of potassium from erythrocytes to the plasma thereby giving a wrong elevated result. Wrong separation and storage time and temperature can lead to deterioration of some blood analytes including potassium (Abdoljalal, 2008). Serum is the most preferred sample for most routine assays in clinical chemistry laboratories (Abdoljalal, 2008). According to Amalia et al. (2021), the average time required to send a serum sample to the laboratory is 2-3 hours. Amalia et al. (2021) also, found no significant difference in serum

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potassium levels with various treatments (directly examined, stored for 2 hours, or stored for 3hour at 4^{o}C).

To the best of our knowledge, there is no documented established study in our environment on the degree of deterioration of serum potassium with respect to storage time and temperature. This study seeks to determine the maximum storage time for serum potassium sample stored at 4^{o}C and -25^{o}C within which an accurate result can be obtained.

Materials and Methods

Study Area

This study was conducted in the Department of Medical Laboratory Science University of Jos, Plateau State in May 2023.

Study Population

The study population comprises students of the Medical Laboratory Science Department, University of Jos, Plateau State.

Ethical Consideration

Ethical clearance was sought and obtained from the Ethics Committee of Jos University Teaching Hospital (JUTH) with reference number JUTH/DCS/IREC/127/XXXI/471.
The consent of the participants to collect their blood samples and publish the result of the research was sought and obtained before enrolment.

Study Design

This was a school-based analytical comparative study.

Sampling Technique

A self-selection sampling design was used for sample collection.

Inclusion Criteria

Apparently healthy individuals of age group 20-40 years were included in this study.

Exclusion Criteria

Critically ill patients, children, lactating and pregnant women were excluded from this study.

Sample Collection and Preparation

Blood samples were collected through venepuncture. Each volunteer was made to sit comfortably on a chair, a tourniquet was applied to the upper arm to make the vein prominent. The area was sterilized with a cotton swab moistened with 70% alcohol. Using a 5ml needle and syringe, about 4mls of blood was withdrawn into the empty sterile plain container. The blood samples were allowed to clot at room temperature for 30 minutes. The samples were centrifuged at 3000rpm for 5 minutes. Samples with visible haemolysis were excluded from the study, serum was separated as soon as possible preferably within two hours of sample collection. Serum K was analysed immediately serving as the control.

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Samples were then divided into different aliquots into cryovials of 2 groups. Each group consists of 6 cryovial serum aliquots (6X12) and were stored at 4°C and -25°C for K analysis at 1hr, 2hr, 4hr, 24hr, 48hr and 72hr.

Research questions

i. What is the effect of temperature on serum potassium level of samples stored at 4°C and -25°C?
ii. What is the effect of storage time serum potassium level of samples stored at 4°C and -25°C?

Methodology

Potassium was estimated by a turbidometric method using TPB Na (Tetraphenylborate sodium) as described by Henry et al. (1974).

$\longrightarrow$ Na^- Tetraphenylborate + K^+ K^- Tetraphenylborate + Na^+

The extent of turbidity is proportional to the potassium concentration and was measured photometrically at 578 nm.

Statistical Analysis

Data was analysed using SPSS version 16. Duncan's multiple range test was used for analysis of the level of significance in K levels at different temperatures and times. The effect of storage temperature and time on serum potassium level was analysed using logistic regression. Test of probability less than 0.05($p<0.05$) was regarded as significant.

Results and Discussion

Demographic Data

Table 1 shows the age and gender characteristics of the study population. Six (50.0%) were males out of 12 participants. Seven (58.3%) were 20-25yrs, 4(33.3%) were 26-30yrs and 1 (8.3%) were 31-35yrs.

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Table 1: Demographic characteristics of the study population

		N	Percent (%)
Age group	20-25yrs	7	58.3
	26 -30	4	33.3
	31-35yrs	1	8.3
	Total	12	100.0
Gender	Males	6	50.0
	Females	6	50.0
	Total	12	100.0

Serum K concentrations at room temperature (25°C)- zero hour (control) and at 4°C by specific time intervals (case)

There was no significant difference ($p>0.05$) in serum K level of samples stored at 4°C from 1hr to 4hrs when compared with the control (0hr) and within the specific time intervals (1hr, 2hr, 4hr, 48hrs and 72hrs). However, there was a slight non-significant ($p>0.05$) rise in K levels from 1 hr to 2 hrs and slight non-significant ($p>0.05$) decrease at 4hrs when compared with the control (0 hr). There was a significant decrease ($p<0.05$) at 24hrs when compared with the control but was non-significant ($p>0.05$) when compared with the level at 4hrs. There was a non-significant rise and fall ($p>0.05$) in K levels at 48hrs and 72hrs respectively when compared with the control (Table 2).

Table 2: Serum K concentrations at room temperature (25°C)- at zero hour (control) and at 4°C by specific time intervals (case)

.

	Time of storage	Mean ±. SD (mmol/l)	N	N
K level at 4°C	0hr (control at 25°C)	$4.8750 \pm .55970^{ab}$		12
	1hr	$4.9592 \pm .55394^{ab}$		12
	2hrs	$4.9408 \pm .71136^{ab}$		12
	4hrs	$4.4692 \pm .41599^{bc}$		12
	24hrs	$3.9875 \pm .56774^{c}$		12
	48hrs	$5.3950 \pm .69773^{a}$		12
	72hrs	$4.5300 \pm .60576^{b}$		12

Each value is a mean of n determinations ± S.D. (n is 12). Values carrying different superscripts along the same column are significantly different ($p<0.05$).
K = potassium

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Serum K concentrations at room temperature (25ºC) -zero hour (control) and at -25ºC by specific time intervals (case)

There was no significant difference (p>0.05) in serum K levels of samples stored at -25ºC when compared with the control (0 hr) and among the specific time intervals from 1hr, 2hr, 4hr, 24hrs to 48 hrs. There was significant decrease(p<0.05) in the K level at 72hrs when compared with the control and other specific time interval from 1hr, 2hr, 4hr, 24hrs to 48hrs (Table 3)

Table 3: Serum K concentrations at room temperature (25ºC) - at zero hour (control) and at -25ºC by specific time intervals (case)

	Time of storage	Mean ± SD (mmol/L)	N	N
K level at-25ºC	0hr (control at -25ºC)	4.8750±.55970[a]		12
	1hr	4.8492±.59129[a]		12
	2hrs	4.8267±.59570[a]		12
	4hrs	4.9950±.55165[a]		12
	24hrs	5.0642±.56739[a]		12
	48hrs	5.1592±.55965[a]		12
	72hrs	4.1775±.55869[b]		12

Each value is a mean of n determinations ± S.D. (n is 12). Values carrying different superscripts along the same column are significantly different (p<0.05).
K = potassium

Overall Mean serum K levels at 4ºC and -25ºC

The overall mean showed that serum K level of samples stored at -25ºC was slightly higher than that of -4ºC though this was not significant (p>0.05) (Table 4)

Table 4: Overall Mean of serum K levels at 4ºC and -25ºC

	Overall Mean (mmol/L)	Std. Deviation	N (12 samples X 7specific time intervals run)	p-value
K level at 4ºC	4.7367	.71016	84	0.184
K level at-25ºC	4.8495	.62434	84	

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Correlation between K levels of samples stored at 4ºC and -25ºC by storage time intervals

There was a non-significant negative correlation between storage time intervals and storage temperatures (4ºC and -25ºC) respectively on the serum K levels (r= -.113, p=.153; r= -.142, p=0.099 respectively). There was a significant positive correlation in serum K levels of samples stored at 4ºC and -25ºC (r=.33, p=.001) (Table 5).

Table 5: Correlation between K levels at 4ºC and -25ºC by storage time intervals

		Time	K_4ºC	K_25ºC
Pearson Correlation	Time	1.000	-.113	-.142
	K at 4ºC	-.113	1.000	.337
	K at 25ºC	-.142	.337	1.000
Sig. (1-tailed)	Time	.	.153	.099
	K at 4ºC	.153	.	.001
	K at 25ºC	.099	.001	.

Effect of temperatures (4ºC and -25ºC) on serum K level at the various time intervals

The effects of temperatures (4ºC and -25ºC) on serum K level at the various time intervals was tested using linear regression analysis (Table 7). There was a non-significant negative effect of storage temperatures (4ºC and -25ºC) on the serum K level at the various time intervals {immediately after collection (0 hr), 1hr, 2hr, 4 hrs, 24hrs, 48hrs and 72hrs of storage} (F= 1.06, p=.306; and F=1.691 ,p=.197) respectively (Table 6)

Table 6: Effect of temperatures (4ºC and -25ºC) on serum K level at the various time intervals

Model	Sum of Squares	Df	Mean Square	F	Sig.
4ºC Regression	.534	1	.534	1.060	.306[b]
Residual	41.325	82	.504		
Total	41.859	83			
-25ºC Regression	1	1	.654	1.691	.197[b]
Residual	31.700	82	.387		
Total	32.354	83			

a. Dependent Variables:K_4ºC and K_25ºC
b. Predictors: (Constant), time

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Comparison on the strength of the effects of the storage temperatures on serum K level

Using the beta function of the model to compare the strength of the effects of 4°C and -25°C on serum K level showed that -25°C has stronger effect (beta =142) on serum K level than 4°C (beta= 113) at the various time interval at 95% confidence interval (Table 7).

Table 7: Comparison on the strength of the effects of the storage temperatures on serum K level

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	4.896	.173		28.269	.000	4.552	5.241
Time	-.040	.039	-.113	-1.030	.306	-.117	.037
(Constant)	5.026	.152		33.132	.000	4.724	5.328
Time	-.044	.034	-.142	-1.300	.197	-.112	.023

a. Dependent Variable: K at 4°C and K at -25°C

Effect of time on serum K level at the storage temperatures

There is a non-significant negative effect of time on the K levels at the two storage temperatures 4°Cand -25°C (beta= -.073, p=0.530 and beta -.117, p= .317 respectively) (Tables 8 and 9)

Table 8: Effect of time on serum K level at the storage temperatures of 4°Cand -25°C

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	8.394	2	4.197	1.038	.359[b]
Residual	327.606	81	4.045		
Total	336.000	83			

a. Dependent Variable: time

b. Predictors: (Constant), K at 25°C, K at 4°C

Table 9: Strength of the effect of time on serum K level at the storage temperatures 4°Cand -25°C

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	6.820	1.972		3.458	.001
K_4°C	-.208	.330	-.073	-.630	.530
K_25°C	-.378	.376	-.117	-1.007	.317

a. Dependent Variable: time

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Discussion

From the result of the study, storing serum samples for K determination at 4^{o}C and -25^{o}C for a maximum of 4hrs and 48 hrs respectively when compared with the control at room temperature of 25^{o}C gives a stable, reliable and reproducible result. However, storage for up to 24hrs and 72 hours at 4^{o}C and -25^{o}C respectively gives a significantly unstable, unreliable, and inaccurate result.

The overall mean showed that the serum potassium level of the sample stored at -25^{o}C was slightly higher than that 4^{o}C. This result is similar to the report of some studies although using different methods of analysis. A study carried out by Amalia et al., in 2021, in Indonesia reveals that there is no significant change in serum potassium level stored at 4^{o}C for 2, 3, and 4 hours. In addition, Vijayasamundeeswari et al., in 2017, in India showed that there is also no significant change in serum potassium levels stored at 4^{o}C for 24 hours, 48 hours, and 72 hours using ion-selective electrodes. Another study carried out by Tayal et al. (2017), in India showed that there is no significant change in the serum potassium level of samples stored at -25^{o}C for 24hours, 48 hours, and 72 hours. Nwosu et al., in 201, in Ebonyi State South-East Nigeria showed that there is no significant change in serum potassium level stored at 4^{o}C for 4hours, 24 hours, 46 hours and so on. The possible reason for its non-significance may be due to the effect of the storage temperatures on the analyte (Vijayasamundeeswari et al., 2017).

Serum potassium is an essential electrolyte that plays a crucial role in various physiological processes, including nerve and muscle function, heart rhythm regulation, and maintaining overall electrolyte balance. Accurate measurement of serum potassium levels is important in diagnosing and managing various medical conditions, thus the effect of storage temperature and time on serum potassium levels among apparently healthy individuals is an important consideration in laboratory medicine. From this study, there was no significant difference ($p>0.05$) in serum K levels of samples stored at 4°C from 1hr to 4hrs when compared with the control (0hr) (Table 2). This means that when comparing the serum potassium levels of samples stored at 4°C for 1 hour, 2 hours, and 4 hours with the control samples analysed at 0 hours, there was no statistically significant difference. Our finding is similar to the report by Amalia et al. (2021). The possible reasons for the non- significant (>0.05) change on serum K level over the stated time intervals could be due to the fact that the samples were stored at a constant temperature of 4°C, which could have minimized any changes in serum potassium levels over the observed time period. It could also be due to small sample size, it might not have been sufficient to detect any significant changes in serum potassium level as was noted by Amalia et al. (2021).

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There was no significant difference ($p>0.05$) in serum K levels of samples stored at -25°C when compared with the control (0 hr) and among the various time intervals from 1 hr to 48 hrs (Table 3). This implies that the potassium levels remained relatively stable during this period. This may be because of storage at -25ºC the cellular membranes of the samples are less prone to damage and disruption, which helps in preserving the normal potassium gradient across the cell membrane. Many enzymes involved in potassium homeostasis are sensitive to temperature changes (Udensi et al., 2017). Lowering the temperature to -25°C might have reduced the activity of the enzymes that could lead to potassium fluctuations, thereby stabilizing serum potassium levels (Udensi et al., 2017). Proteins play a crucial role in maintaining potassium balance within cells. At lower temperatures, protein denaturation and degradation are minimized, ensuring the stability of proteins involved in potassium transport and regulation. Potassium ions move across cell membranes through specific ion channels. These channels may be less active or more stable at lower temperatures, leading to consistent potassium levels (Udensi et al., 2017).

There was a significant decrease ($p<0.05$) in the K level at 72 hrs when compared with the control and other time intervals (1 hr, 2 hr, 4 hr, 24 hr, 48 hr, and 72 hr). At the 72-hour mark, there was a significant ($p<0.05$) decrease in potassium levels when compared to the control sample and all the other time intervals mentioned (1 hour, 2 hours, 4 hours, 24 hours, and 72 hours). The difference was statistically significant, indicating a notable drop in potassium levels after 72 hours of storage at -25°C. The possible reason(s) could be that some biological materials may contain enzymes that can remain active at low temperatures, leading to degradation or changes in the sample over time (Udensi et al., 2017). While low temperatures can slow down microbial activity, contamination by microorganisms during storage could result in observed decrease as was stated by Nwosu et al. (2011).

The result suggests that storing serum samples at -25°C for up to 48 hours did not significantly affect potassium levels. However, there was a slight non-significant ($p>0.05$) decrease from 1 hour to 2 hours and a non-significant ($p>0.05$) increase from 4 hours to 48 hours. After 72 hours of storage, there was a significant decrease ($p>0.05$) in potassium levels compared to both the control and other time intervals. This implies that storing serum samples for K determination up to or beyond 72 hrs may significantly affect the result. There was a non-significant negative correlation in serum K levels between storage time intervals and storage temperatures (4°C and -25°C). This suggests that the observed correlation is likely due to chance. There was a significant positive correlation in serum K levels between 4°C storage temperature and -25°C ($r=.337$, $p=.001$). This implies that temperature has a significant positive influence on serum K level.

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There was a non-significant negative effect of storage temperatures (4^{o}C and -25^{o}C) on the serum K level (F= 1.06, p=.306; and F=1.691, p=.197; Table 7). The beta values represent the estimated coefficients for the effect of temperature on the K levels (Table 8). In this case, the beta values are -0.113 for 4°C storage and -0.142 for -25°C storage and the p-value for 4^{o}c and -25^{o}c are-0.073 and -117 respectively for the effect of time on K level at the respective storage temperature (Table 8 and Table 9). These negative values suggest a potential decrease in K levels over time, but they are not statistically significant. Therefore, based on these results, it can be concluded that there is no significant negative effect of time on the K levels at both 4°C and -25°C storage temperatures. In other words, the available evidence does not support the idea that time has a meaningful impact on the K levels under these storage conditions.

When blood samples are collected for laboratory analysis, they need to be properly handled and stored to ensure accurate results. Though serum samples were used in this study, very small level of RBCs that may not be visible to the naked eye may be present in the serum as was noted by Tayal et al. (2017). This may affect the serum K level on storage. To ensure accurate measurements, it is generally recommended to process blood samples and perform potassium analysis promptly after collection (Kathrina et al., 2023).

Conclusion

Storing serum samples for K determination at 4^{o}C and -25^{o}C for a maximum of 4hrs and 48 hrs respectively gives a stable, reliable and reproducible result. However, storage for up to 24hrs and 78 hours at 4^{o}C and -25^{o}C respectively gives a significantly unstable, unreliable and inaccurate result. This implies that serum samples for K determination should be assayed immediately and in the case of storage, it should not exceed 4 hrs and 48 hrs at 4^{o}C and -25^{o}C respectively. This will help in producing an accurate result that is a true representation of the patient's condition thus improving the diagnosis and proper management of patients with K – related disorders thereby reducing morbidity and mortality.

Limitations of the study

1. Due to the practical and financial constraints, only a relatively small number of specimens were tested, however analysis of larger number of samples could be of further help.

2. Analysis of other electrolytes such as sodium, chloride, or even bicarbonate can be carried out for broader knowledge in the effect by storage temperature and time.

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Conflict of interest
The authors declare that there is no conflict of interest.

Authors' Contribution
CAO conceptualized the study, supervised it, validated the methods and results, involved in statistical analysis and interpretation, involved in editing and reviewing the manuscript. NSG was involved in data collection, sample collection, write–up of the original draft.

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