

Evaluation Of The Impact Of Storage Temperature On Glucose Level Of Blood Samples Among Students Of Federal College Of Medical Laboratory Technology (Science), Jos Plateau State

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The work was carried out in collaboration among all authors, authors AHA, IEI, NLL, EMI, AUS, ON, and MJ did the laboratory analysis, JNI, KNI did the literature review and reading, authors NBC, and DMB did the statistical analysis and blood sample collections. All authors read and approved the final manuscript

Abstract- Background: Glucose is the main energy source for humans. The nervous system including the brain completely depends on the glucose for energy. Glucose is considered to be one of the most important parameters in the routine analysis and monitoring of disease such as diabetes mellitus. Aim: An environment design is drowned, aimed at evaluating the impact of storage temperature on glucose level of blood sample (plasma and serum) among students of Federal College of Medical Laboratory Science and Technology, Jos Plateau State. Methodology: The total of fifty (50) randomly selected apparently healthy students of Federal College of Medical Laboratory Technology (Science), Jos aged 18 to 28 years were recruited as subjects into this study. Fasting blood samples were collected from each of the subjects into plain and sodium fluoride oxalate bottles from serum and plasma respectively. The samples were spun and separated to obtain the baseline value and therefore after a specified time interval across 72hours. The plasma and serum samples from each patient were analyzed for glucose concentration immediately after separation to obtain the baseline value. The plasma and serum sample were then divided into two equal parts; one part was kept in a refrigerator at 4oC while the other part was kept at -20oC at freezer temperature. Further analyses of the samples kept at 4oC were carried out after 2, 4 and 6 hours. Sample stored at -20OC were analyzed after 24, 48, and 72hour after separation respectively. The serum and plasma glucose were determined by glucose oxidase colorimetric assay kits obtained from Randox Laboratory Limited United Kingdom. Data were analyzed using student t-test and performed using the statistical package for social science (SPSS) version 20.2. Results: results obtained show the mean and standard deviation of a paired sample for plasma and serum. In plasma, it shows that the mean +S.D of 2, 4 and 6 hours at 4oC (7.57+1.70, 5.40+1.49 and 7.84+0.73 respectively) were statistically significant when compared to the baseline (0hour) value (4.74+0.56). The baseline and 72hours, -20oC (4.74+0.56 and 4.49+0.49 respectively) were significantly lower ($P < 0.05$) than that of 24 and 48 hours, -20oC (4.79+0.58 and 4.90+0.93 respectively). In serum, similar results were obtained. Conclusion: In conclusion, the result of this study shows the stability of plasma serum glucose stored at 20oC is lost within 72hours of storage.

Index Terms- Blood Glucose, Diabetes mellitus, Hyperglycemia, Storage temperature of glucose

I. INTRODUCTION

Glucose is the main energy source for humans. The nervous system including the brain completely depends on the glucose from the surroundings extracellular fluids for energy.

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Nervous systems tissue does not have the ability for carbohydrate storage; therefore it is important to maintain a steady supply of glucose to the tissue. When carbohydrate falls below a certain level, the nervous tissues loses the primary energy sources and are incapable of maintaining normal functions (Bishop, et al, 2010).

Specimen collections and handling can result in inaccurate test results, which can lead to the mismanagement of a patient. Some of the most common errors include clinical errors, hemolysed samples and incorrect use of anticoagulant, hemolysis from a venipuncture can lead to erroneous results for an array of analysis (Kitchens, 2006).

The temperature and the time at which a sample is stored can have a great influence on prescription drug concentration, analytes values and enzymes stability in the blood sample.

For instance (Bennetto et al, 2004), proved that variable temperature and length of storage time can alter the storage of neviraprine, a highly prescribed HIV treatment analytes values as creatinine, phosphorus, potassium, aspartate, aminotransferase and alanine aminotransferase in blood samples can also be affected by variables such as temperature (Rehak and Chiang 1988).

Blood samples kept in serum and plasma at 25°C has significant changes in nine of the blood profile tests included in the comprehensive metabolic panel. Glucose, potassium, creatinine, total protein, calcium, albumin, and CO² and all affected by storage duration factor (Boyanton and Blick, 2002).

The effects of blood storage time on the accuracy of comprehensive metabolic plasma result were investigated by (Jessica and Kitchens, 2006), and concluded a significant change in parameters result including glucose.

II. RATIONALE:

Blood glucose estimation has always been an easy and productive means for the diagnoses and short term production of diabetes complications and hypoglycemia (Nkereuwem, S.E. et al 2020).

In America alone 1.5 million individuals, are diagnosed with diabetes every year (ADA, 2017). In a developing country like ours, laboratory personnel's are constantly faced with the problems of inconsistent power supply such that specimens for analysis are left on the bench at ambient temperature for several hours or even days before analysis can be done.

The study aims to investigate the changes in glucose levels in plasma and serum samples stored at 4°C and -20°C.

In Nigeria there are no published study concerned with the impact of storage temperature or duration on blood glucose in serum and plasma.

The concentration of blood glucose is regulated by multiple pathways with multiple hormones (Said Orably, 2013). During fasting state; a progressive decline in blood glucose concentration is stopped by glycogenolysis in the liver. A small amount of glucose also may be derived from synthesis within the kidneys. The organ possesses the enzymes which are necessary to produce glucose by gluconeogenesis and glycogenolysis (Ibanga, I. E. et al, 2023).

The concentration of glucose in the blood is normally maintained within a narrow interval by hormones that modulates the movement of glucose within the body, they include insulin which decreases blood glucose and the counter regulatory hormones (glucagon, epinephrine, cortisol and growth hormones), (Cart et al, 2018).

In clinical hypoglycemia, plasma or serum glucose concentration is low enough to cause symptoms and or signs including impairment of brain functions (Philip Cryer et al, 2009).

In healthy individuals, symptoms of hypoglycemia develop at a mean plasma glucose concentration of approximately 55mg/dl (3.0 mmol/l) (Cryer 2001).

The results of blood glucose determination can be strongly affected by the methods of storage and handling of the blood samples between the time of collection and the time of analysis (Young and Bermes, 1999), improperly handled or stored samples could generate results that may mislead the clinicians into wrong judgment / diagnosis (Clark et al, 2003), Attention to proper blood handling/ processing and storage procedure and avoidance of hemolysis are important in blood clinical analysis and in the proper interpretation of experimental results (Morris et al, 2002). Different storage times were also found to be contributing to a significant reduction in neonatal blood glucose levels along with the length of storage duration (Xiao-liZhu et al, 2017).

The effective of sodium fluoride as glycolysis inhibitors on blood glucose measurement was questioned. The analysis of paired glucose samples with and without antiglycolytic agent was needed to conclude a significant reduction in the delayed plasma glucose concentration (Lee et al, 2009).

III. METHODS:

Study Design:

A total of fifty (50) apparently healthy students of Federal College of Medical Laboratory Technology (Science), Jos were recruited into this study.

Fasting blood samples were collected from each subject into plain and fluoride oxalate bottles for plasma and serum respectively.

The plasma and serum samples from each subject were analyzed for glucose concentration immediately after separation to obtain the baseline levels. The plasma and serum samples were then divided into equal parts; one part was kept in a refrigerator of 4°C while the other part was kept in a freezer at -20°C temperature. Further analysis of the samples kept at the refrigerator were analyzed after 2, 4 and 6 hours, samples stored in freezer at -20°C were analyzed after 24, 48 and 72 hours after separation respectively.

Subject Selection:

Subjects were randomly selected among the volunteer students of FCMLT, JOS. There were 20 apparently healthy females and 30 apparently healthy males aged between 18 and 28 years. Individuals on any medications were excluded from the study, all blood samples were collected after obtaining written consent from the participants. Blood drawn was performed according to the standard operating procedures.

Ethical Consideration:

Ethical approval for the use of human subjects for research was sought and obtained from the research ethics – laboratory committee of FCMLT, Jos.

Laboratory Analysis

Blood sampling and processing:

6mls of fasting venous blood samples was collected from each enrolled subject into each plain and fluoride oxalate bottles for serum and plasma respectively. The blood samples were dispensed into each of the following collection tube;

- a. Half (3ml) was added into plain container, allowed to stand at room temperature for 15 minutes to clot, after which it was centrifuged at 300rpm for 5minutes, and the serum from it was separated into another plain container
- b. The other half was added into a container with suitable quality of fluoride oxalate, anticoagulant, and centrifuged immediately after collection at 300rpm for 5minutes and the plasma from it was dispensed into a plain container.

Estimation of blood glucose

Blood glucose was determined by glucose oxidase colorimetric assay kit obtained from Randox Laboratory Limited United Kingdom.

Data Analysis

The statistical analysis was performed using the statistical package for social sciences (SPSS) Version 20.0. Student's t-test was used to study the significance of the difference in the means. P-value of less than 0.05 was considered statistical significant.

IV. RESULT:

Impact of storage temperature of 4°C and -20°C on glucose levels of plasma and serum samples at 0hour, 2hours, 4hours, 6hours, 24hours, 48hours and 72hours intervals

Result: paired samples statistics

Table 1:
PAIRED SAMPLES STATISTICS (Table 1)

Parameters	Mean / Std. Deviation	No. of Samples (N)	Std. Error Mean
Plasma	4.74±0.561	50	0.177
(0- Hour) Serum	4.50±0.571	50	0.180

Plasma (2- Hours,4°C) Serum	7.57±1.702	50	0.538
	7.36±1.452	50	0.459
Plasma (4-Hours,4°C) Serum	5.40±1.497	50	0.473
	4.98±1.213	50	0.384
Plasma (6-Hours,4°C) Serum	7.84±0.736	50	0.233
	7.81±0.751	50	0.238
Plasma (24-Hours,-20°C) Serum	4.79±0.587	50	0.186
	4.74±0.683	50	0.216
Plasma (48-Hours,-20°C) Serum	4.90±0.934	50	0.295
	4.88±0.977	50	0.309
Plasma (72-Hours,-20°C) Serum	4.49±0.475	50	0.150
	4.48±0.473	50	0.150

Table 1: Shows the mean and standard deviation of paired samples for storage temperatures (4°C & -20°C), is 4.74 ± 0.56 , 7.57 ± 1.70 , 5.40 ± 1.49 , 7.84 ± 0.73 and 4.79 ± 0.58 , 4.90 ± 0.93 , 4.49 ± 0.47 were gotten for 0,2,4,6,24,48 and 72 hours respectively and in serum (4°C & -20°C), 4.50 ± 0.57 , 7.36 ± 1.45 , 4.98 ± 1.21 , 7.81 ± 0.75 and 4.74 ± 0.68 , 4.88 ± 0.97 , 4.48 ± 0.47 were gotten for 0,2,4,6,24,48 and 72 hours respectively.

Table 2:
PAIRED SAMPLES CORRELATIONS (Table 2)

Parameters	No. of Samples	Correlation	Significant (P-values)
Plasma/Serum (0-Hour)	50	0.760	0.011
Plasma/Serum (2-Hour,4°C)	50	0.994	0.000
Plasma/Serum (4-Hours,4°C)	50	0.978	0.000
Plasma/Serum (6-Hours,4°C)	50	0.970	0.000
Plasma/Serum (24-Hours,-20°C)	50	0.976	0.000
Plasma/Serum (48-Hours,-20°C)	50	0.988	0.000
Plasma/Serum(72-Hours,-20°C)	50	0.979	0.000

FROM TABLE 2

There is a correlation between Plasma and Serum at 0 hour as indicated by positive correlation coefficient of 0.760 with p-value of 0.011. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 2 hours, 4 °C as indicated by positive correlation coefficient of 0.994 with p-value of 0.000. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 4 hours, 4 °C as indicated by positive correlation coefficient of 0.978 with p-value of 0.000. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 6 hours, 4 °C as indicated by positive correlation coefficient of 0.970 with p-value of 0.00.0 Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 24 hours, -20 °C as indicated by positive correlation coefficient of 0.976 with p-value of 0.000. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 48 hours,-20 °C as indicated by positive correlation coefficient of 0.988 with p-value of 0.000. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

There is a correlation between Plasma and Serum at 72 hours, -20 °C as indicated by positive correlation coefficient of 0.979 with p-value of 0.000. Since the p-value < 0.05, the positive relationship existing between plasma and serum is statistically significant.

Table 3:
PAIRED SAMPLES TEST (TABLE 3)
Paired Differences

Parameters	Mean / Std. Deviation	Std. Error Mean	Lower	Upper	"t"	df	Sig.(2-Tailed)
Plasma/Serum (0-Hour,4°C)	0.237±0.392	0.124	-0.044	0.518	1.911	49	0.088
Plasma/Serum (2-Hours,4°C)	0.213±0.305	0.096	-0.005	0.431	2.210	49	0.054
Plasma/Serum (4-Hours,4°C)	0.413±0.399	0.126	0.127	0.699	3.269	49	0.010
Plasma/Serum (6-Hours,4°C)	0.030±0.182	0.058	-0.100	0.160	0.520	49	0.615
Plasma/Serum (24-Hours,- 20°C)	0.047±0.170	0.054	0.074	0.168	0.877	49	0.403
Plasma/Serum (48-Hours,- 20°C)	0.018±0.152	0.048	-0.091	0.127	0.375	49	0.717
Plasma/Serum (72-Hours,- 20°C)	0.006±0.098	0.031	-0.064	0.076	0.194	49	0.851

Result report shows that mean standard deviation of paired samples statistics N number of samples, significant at P ≤0.05, unit of measurement of glucose is in mmol/l.

FROM TABLE 3

There is no significance difference in the mean difference of plasma and serum (0-Hour, 4 °C) as measured by paired t-test, t (49) = 1.911 with p -value = 0.088. Since p>0.05, there is no sufficient evidence to show statistically significance difference.

There is no significance difference in the mean difference of plasma and serum (2-Hours, 4 °C) as measured by paired t-test, t (49) = 2.210 with p -value = 0.054. Since p>0.05, there is no sufficient evidence to show statistically significance difference.

There is a significance difference in the mean difference of plasma and serum (4-Hours, 4 °C) as measured by paired t-test, t (49) = 3.269 with p -value = 0.010. Since p<0.05, there is sufficient evidence to show statistically significance.

There is no significance difference in the mean difference of plasma and serum (6-Hours, 4 °C) as measured by paired t-test, $t(49) = 0.520$ with p -value = 0.615. Since $p > 0.05$, there is no sufficient evidence to show statistically significance difference.

There is no significance difference in the mean difference of plasma and serum (24-Hours, -20 °C) as measured by paired t-test, $t(49) = 0.877$ with p -value = 0.403. Since $p > 0.05$, there is no sufficient evidence to show statistically significance difference. There is no significance difference in the mean difference of plasma and serum (48-Hours, -20 °C) as measured by paired t-test, $t(49) = 0.375$ with p -value = 0.717. Since $p > 0.05$, there is no sufficient evidence to show statistically significance difference. There is no significance difference in the mean difference of plasma and serum (72-Hours, -20 °C) as measured by paired t-test, $t(49) = 0.194$ with p -value = 0.851. Since $p > 0.05$, there is no sufficient evidence to show statistically significance difference.

V. DISCUSSION

Glucose is considered to be at the most important parameter in routine analysis and monitoring for disorder such as diabetes methods. This study was primarily designed to estimate glucose concentration in healthy study of Federal College of Medical Laboratory Technology (Science), Jos in an attempt to spot the impact of storage temperature and duration on serum and plasma glucose levels. The study showed a significant reduction in plasma and serum glucose at (-20°C) temperature within the seventy two hours of storage (P value < 0.05) and this result disagrees with (Nwosu and Nwani, 2008) and (Odozo et al, 2012) indicating in stationary of glucose creation in plasma and serum after two hours of storage at 40C temperature, and disagrees with (Manjani, 2006) who after 72hour of storage of -20°C.

For plasma and serum, the study proved that plasma and serum stored at -20°C at 24 and 48hours loses it strongly (P . value < 0.05) and the result disagrees with (Nwosu and Nwani, 2008).

VI. CONCLUSION

The plasma and serum glucose stored at 4°C was found to be sensitive to delay within 2, 4 and 6 hours, while the sensitivity for plasma and serum glucose stored at -20°C within 72hours of storage. The plasma and serum glucose was found to be stable at -20°C for 72hours of storage.

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