Journal of Advances in Medical and Pharmaceutical Sciences

Volume 25, Issue 1, Page 23-30, 2023; Article no.JAMPS.97077
ISSN: 2394-1111

The Impact of Delayed Separation of Plasma and Serum Sample on Glucose Parameter of Apparently Healthy Students of Federal School of Medical Laboratory Technology (Science), Jos, Nigeria

Ukeme Sambo Abara [a*], Imoh Etim Ibanga [a], Lucy Larrycarr Nwapi [a], Matina Imaobong Ekpo [a], Hilary Andrew Akpan [a], Obinna Nkechi [a], John Okon Mkpani [a], Nduese Innocent Joseph [a], Nanle Ibrahim Konglam [a], Brendan Chukwueke Nkwocha [a] and Manji Bala Dalen [a]

[a] Federal School of Medical Laboratory Technology (Science), Jos, Plateau State, Nigeria.

Authors' contributions

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

Article Information

DOI: 10.9734/JAMPS/2023/v25i1595

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97077

Original Research Article

Received: 18/12/2022
Accepted: 23/02/2023
Published: 08/03/2023

**Corresponding author: E-mail: jollyabara@yahoo.com;*

ABSTRACT

Background: Estimation of glucose is very important in clinical diagnosis of diabetes mellitus, hyperglycemia and hypoglycemia. The core aim of clinical laboratory service is to produce accurate, precise and reliable test results. The ability of laboratories to do this lies in the availability of well trained personnel, equipment, electricity etc. There has been a progressive increase in the prevalence of diabetes mellitus in Nigeria and the burden is expected to increase even further and the need for proper diagnosis cannot be overemphasized.

Aim: In this study we have tried to evaluate the impact of delayed separation of plasma and serum from cells and clot on glucose level.

Method: A total of fifty (50) randomly selected apparently healthy students of Federal School of Medical Laboratory Technology (Science), Jos were recruited as subject into this study. Blood samples were collected from each of the subjects into plain and anticoagulant bottles for serum and plasma respectively. An aliquot was analyzed within 10 minutes to obtain a baseline value where other values after timely delays were compared. The samples were spun but not separated from the cells/clot all through the period of the analysis. Blood glucose was determined by glucose Oxidase Colourimetric assay kit obtained from Randox Laboratories Limited United Kingdom. Data were analyzed using student's t-test and performed using the Statistical Package for Social Sciences (SPSS) version 20.0.

Results: Result obtained shows the mean and standard deviation of all timing for plasma and serum. In plasma it shows that the means±SD of 2, 4 and 24hours (5.2±0.7, 5.1±0.4 and 5.3±0.5 respectively) were not significantly varied when compared to the baseline (0 hour) value (5.3±0.5). That of baseline and 24 hours (5.3±0.5 and5.3±0.5 respectively) were significantly higher (P<0.001) than that of 48 and 74 hours (1.0±0.20 and 7±0.2 respectively). In serum, similar results were obtained.

Conclusion: In conclusion, where possible, it is advised that the established best practices in processing and analysis of samples be adhered to. However, the results of this work shows reasonably stable results for blood glucose determination could be obtained within 24 hours. Therefore, instead of discarding the sample, it might seem more appropriate that the sample be analyzed and the result reported indicating the number of hours delayed within 24hours.

Keywords: Preanalytical error; blood glucose; diabetes mellitus; hyperglycemia; delayed sample separation.

1. INTRODUCTION

Estimation of glucose is very important in clinical diagnosis of diabetes, hyperglycemia, hypoglycemia and normoglycemia [1]. The core aim of clinical laboratory service is to produce accurate, precise and reliable test results. The ability of laboratories to do this lies in the availability of well trained personnel, equipment, electricity etc. In Nigeria for instance, it is not arguable that there is a good number of well trained Medical Laboratory Personnel to man our medical laboratories but these personnel are grossly unemployed or under employed leading to their mass movement abroad for greener pasture and leaving the few employed ones overwhelmed with work load in the laboratories with the attendant delay in turnaround time, quality of results and poor health outcomes [2]. Again, in Nigeria as well as other resource deficient countries there is still a big challenge of no/poor electricity in many urban, sub-urban and rural settlements and lack of essential laboratory equipment such as centrifuge in many sub-urban and rural areas therefore making timely processing and testing of samples near impossible in those areas [3]. Again, the use of satellite collection centers such as the central collection centers in the outpatient departments (OPD) in collecting samples also result in delays of sample separation due to transportation challenges. The results of blood glucose determinations can be strongly affected by the delay in separation of samples which ultimately results in the delay in the analysis [4,5].

The burden of diabetes is growing in Africa. It is estimated that the African region will have the greatest percentage increase (143%) in the burden of diabetes between 2019 and 2045. Similarly, there has been a progressive increase in the prevalence of diabetes mellitus in Nigeria. Studies have reported prevalence rates of diabetes mellitus ranging between 0.8-4.4% in

some rural communities of Nigeria, while the prevalence in urban areas has been reported to range between 4.6-7%. A recently published systemic review and meta-analysis of studies on the prevalence of diabetes mellitus amongst Nigerians has reported an overall pooled prevalence of 5.77%. As at 2019, 8.2 million Nigerians were estimated to have impaired glucose tolerance, with the number projected to increase to 11.5 million by 2030 [6-8].

Since blood glucose test is very useful in the diagnosis of diabetes mellitus [9,10], it is very important that laboratories produce accurate and timely results for proper and timely management of the patients. The results of blood glucose determinations can be strongly affected by the method of storage and handling of the blood samples between the time of collection and the time of analysis. The major challenge faced by clinical laboratories worldwide is ensuring the integrity and reliability of laboratory results but as analytical variations are being reasonably minimized by the development of new techniques, the relative contribution of pre-analytical factors to spurious results has become a more dominant element in overall test variability [11].

A preanalytical factor of great importance in glucose testing is glycolysis ex vivo. According to the recommendation of the World Health Organization [12], the diagnostic sample for blood glucose determination must be venous plasma placed in ice-water slurry after collection, and separation from cells needs to be done before 30 minutes [13], otherwise, glycolysis will cause decreased glucose results with respect to their in vivo value. Elimination of glycolysis is therefore said to be essential in order to obtain reliable glycaemic results for diagnosis, since it introduces an unpredictable negative bias [14]. Therefore standard operating procedures have been adopted and this is that plasma glucose estimation should be performed within two hours after the collection of sample, and sample preparation (separation of plasma from cells) for the analysis should be performed within one hour of collection [15].

In Nigeria, our laboratories are constantly faced with the problems of inconsistent power supply such that specimens for analysis are left on the laboratory bench at ambient temperature for several hours or even days before light is restored or alternative source of power is gotten for the analysis to be done [16]. In this study, it was aimed that the impact of delayed separation of plasma and serum from the cells and clot on the glucose level of the samples would be clearly determined.

2. METHODS

2.1 Study Design

A cross sectional analytical design was used in this study. A total of fifty (50) subjects who were apparently healthy students of Federal School of Medical Laboratory Technology (Science) (FSMLT), Jos were recruited into this study. Fasting blood samples were collected from each of the subjects into plain and Fluoride Oxalate bottles for serum and plasma extraction respectively. An aliquot was analyzed to obtain a baseline value and this represents the value at ‘zero’ hour where other values after timely delays were compared.

2.2 Subject Selection

Subjects were randomly selected among the volunteer students of FSMLT, Jos. They were 20 apparently healthy males and 30 apparently healthy females aged between 20 and 30years. All blood samples were collected after obtaining written consent from the participants. Blood drawing was performed according to the standard operating procedures.

2.3 Laboratory Analysis

2.3.1 Sample collection and preparation

Four milliliters of fasting blood sample was collected from each subject into each of plain and fluoride oxalate bottles for serum and plasma extraction respectively. Samples were spun but were not separated from the cell/clot all through the period of the analysis and were stored at room temperature all through the period of the analysis. Room temperature in Jos the Plateau State Capital, Nigeria in August 2022 where and when then laboratory analysis was done ranged from 62-76^{o}F or 16.6-24.4^{o}C [17].

2.3.2 Determination of blood glucose

Blood glucose was determined by Glucose Oxidase Colourimetric assay kit obtained from Randox Laboratories Limited United Kingdom with normal (reference) value of 4.2-6.4 mmol/l or 75-115 mg/dl in fasting Serum and plasma samples [18]. However, many hospitals in the study location use 3.5-5.5mmol/L as reference range.

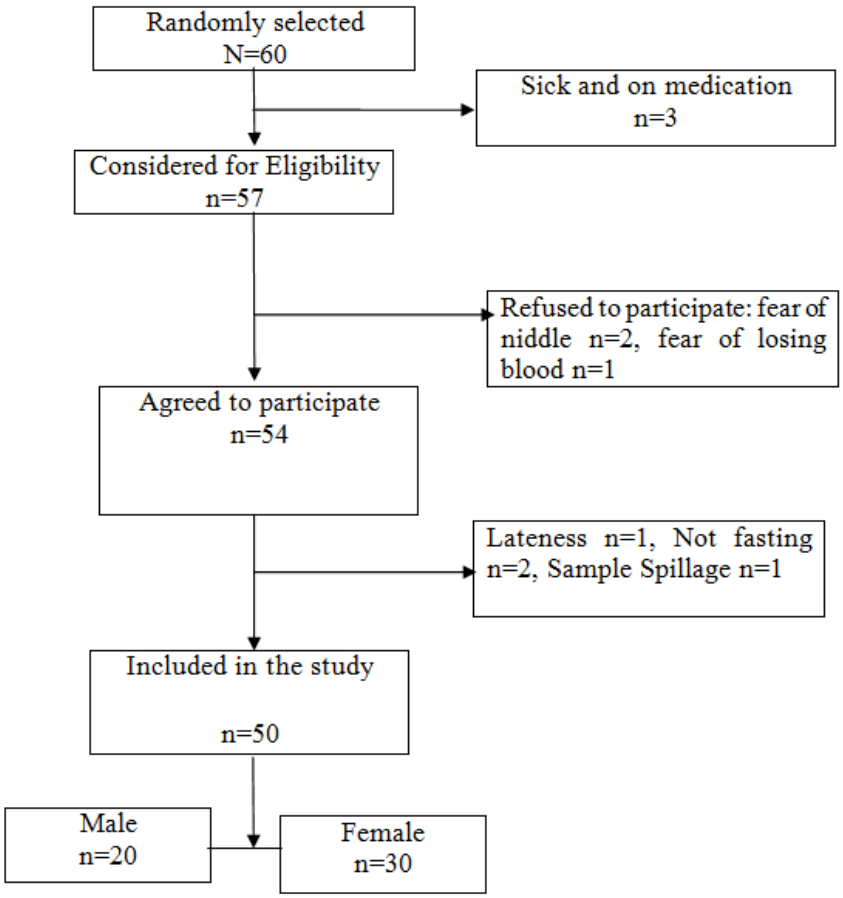

Fig. 1. Flow chart showing the cross sectional analytical design of the study

2.4 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 statistically significant.

3. RESULTS

3.1 Impact of Delayed Separation on Glucose Level of Serum and Plasma Samples

Table 1, Fig. 2 and 3 Show the mean and standard deviation of all timing (delayed separation) for plasma and serum. In plasma, 5.3±0.5, 5.2±0.7, 5.1±0.4, 4.6±0.7, 5.3±0.5, 1.0±0.2, 0.7±0.2 were gotten for 0, 2, 4, 6, 24, 48 and 72 hours delays respectively and in serum, 4.4±0.9, 4.7±0.6, 4.5±0.5, 4.3±0.4, 4.4±0.9, 0.6±0.3, 0.4±0.2 were gotten for 0, 2, 4, 6, 24, 48 and 72 hours delays respectively.

Table 2 describes the impact of two and twenty four hourly delayed separation of plasma from whole blood. It shows that the means±SD of 2, 4 and 24hours (5.2±0.7, 5.1±0.4 and 5.3±0.5 respectively) were not significantly varied when compared to the baseline (5.3±0.5). Again, those of baseline and 24 hours (5.3±0.5 and5.3±0.5 respectively) were significantly higher (P<0.001) than those of 48 hours and 74 hours (1.0±0.20 and 7±0.2 respectively) and that of 48 hours was significantly higher (1.0±0.2, P=0.045) than that of 72 hours (0.7±0.2).

Table 3 describes the impact of two and twenty four hourly delayed separation of serum from clotted blood. It shows that there was no statistical significant variation in the means±SD of 2, 4, 6 and 24hours (4.7±0.6, 4.5±0.5, 4.3±0.4 and 4.4±0.9 respectively) compared to the baseline (4.4±0.9). There was statistically significant variation in the means±SD when 48 and 72 hours (0.6±0.3 and 0.4±0.2 respectively) were compared with the baseline and 24hours (4.4±0.9 and 4.4±0.9 respectively), (P<0.001).

Table 1. Mean and standard deviation of all timing (delayed) for both plasma and serum

Time of Delay	Plasma			Serum		
	N	Mean	SD	N	Mean	SD
0 hour	50	5.3	0.5	50	4.4	0.9
2 hours	50	5.2	0.7	50	4.7	0.6
4 hours	50	5.1	0.4	50	4.5	0.5
6 hours	50	4.6	0.7	50	4.3	0.4
24 hours	50	5.3	0.5	50	4.4	0.9
48 hours	49	1.0	0.2	50	0.6	0.3
72 hours	49	0.7	0.2	50	0.4	0.2

*Result reported as Mean±Standard Deviation, N: Number of samples, *Significant at p≤0.05, Unit of measurement of glucose is in mmol/L*

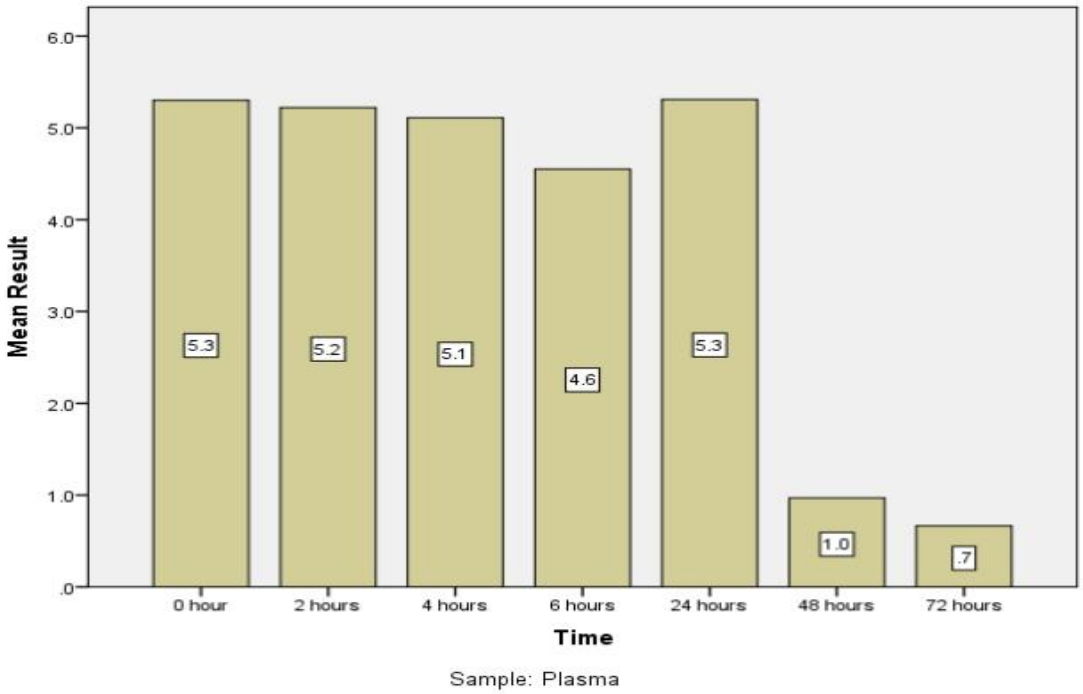

Fig. 2. Bar chart of the mean values of the timed delays in plasma samples

Table 2. Timed delay comparison in plasma sample

Time of Delay	Mean 1	Mean 2	*p* value
0 hour (Mean 1) & 2 hours (Mean 2)	5.3±0.5	5.2±0.7	0.985
2 hours (Mean 1) & 4 hours (Mean 2)	5.2±0.7	5.1±0.4	0.929
4 hours (Mean 1) & 6 hours (Mean 2)	5.1±0.4	4.6±0.7	<0.001*
0 hour (Mean 1) & 24 hours (Mean 2)	5.3±0.5	5.3±0.5	1.000
24 hours(Mean 1) & 48 hours (Mean 2)	5.3±0.5	1.0±0.2	<0.001*
48 hours (Mean 1) & 72 hours (Mean 2)	1.0±0.2	0.7±0.2	0.045*

*Result reported as Mean±Standard Deviation, *Significant at p≤0.05, Number of samples for plasma= 49, others = 50. Unit of measurement of glucose is in mmol/L*

4. DISCUSSION

This study was done to determine the impact of delayed separation of sample on the concentration of glucose in serum and plasma samples. The present study observes that even though it is the best practice to separate sample from cells as soon as it is clotted (for serum) and as soon as it is collected (for plasma), glucose measured from unseparated sample at room temperature could give a reasonable insignificantly varied result within twenty four hours. There may be some unavoidable situation that leads to delay/inability to separate and

analyze the sample. Unavailability of the equipment and electricity as is obtained in some rural settings and hard to reach areas, equipment damage or spoilage may be some of the reasons for delayed separation and analysis of samples.

The outcome of this study shows clearly a remarkable reduction in the concentration of glucose in the sample delayed to be separated beyond twenty four hours. Surprisingly, it is one of the few studies that shows that the glucose could be stable in the samples (both plasma and serum) left unseparated within twenty four hours and this is indicated by the insignificant variation in the levels of the analyte when 0, 2, 4, 6, and 24 hours were compared. Effect of delay in Analysis on Plasma Glucose Concentration was recently studied by Dharmasena et al. [19] and reported a statistically significant change in glucose concentration between the timepoints of one and five hours and three and five hours, however, inconsistent with this present study.

A previous study, [20] Chan et al. stated that delayed separation is one cause of the lack of reproducibility of OGTTs but failed to clearly determine the extent of delays that cause a significant change. Again [21] in their study reported a result inconsistent with this present study, that the decline in serum glucose concentration for all samples stored at 25°C was found to be statistically significant with the Mean glucose value of 1 hour statistically different from baseline value and that at 25°C, serum glucose concentration was found to be decreasing with mean of 10.8% per hour. One of the studies, [22] Jangam et al. observed that delays of 15 min or more in analysis reduce clinical accuracy below the ISO 15197:2003(E) recommendation of 95% and that in their study the accuracy was less than 65% for delays of 60 min.

Table 3. Timed delay comparison in serum sample

Time of Delay	Mean 1	Mean 2	*p* value
0 hour (Mean 1) & 2 hours (Mean 2)	4.4±0.9	4.7±0.6	0.320
2 hours (Mean 1) & 4 hours (Mean 2)	4.7±0.6	4.5±0.5	0.875
4 hours (Mean 1) & 6 hours (Mean 2)	4.5±0.5	4.3±0.4	0.530
0 hour (Mean 1) & 24 hours (Mean 2)	4.4±0.9	4.4±0.9	1.000
24 hours (Mean 1) & 48 hours (Mean 2)	4.4±0.9	0.6±0.3	<0.001*
48 hours (Mean 1) & 72 hours (Mean 2)	0.6±0.3	0.4±0.2	0.907

*Result reported as Mean±Standard Deviation, *Significant at p≤0.05. Unit of measurement of glucose is in mmol/L*

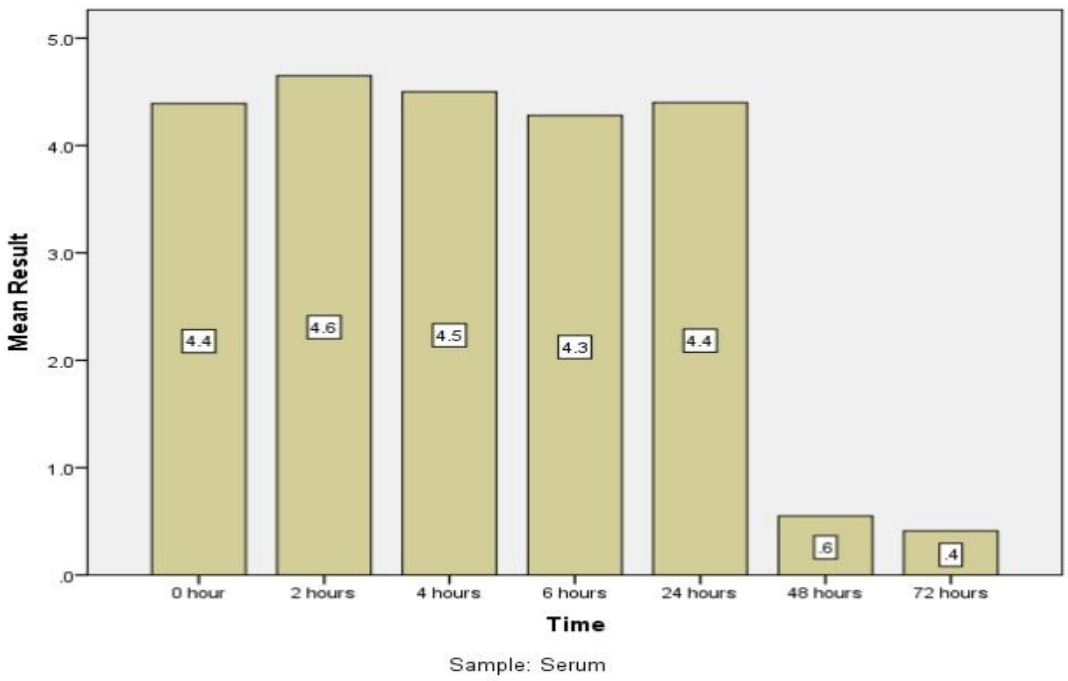

Fig. 3. Bar chart of the mean values of the timed delays in serum samples

The relative stability of the glucose in these samples up to 24 hours as is reported in this study is not similar with the results of the study of the other authors [19-22] as stated above but seems to be in line with findings of Mingoas et al. [23]. The relative low atmospheric (room) temperature in the study location which sometimes are as low as the 17^{o}C among other things may be responsible for the stability of the glucose in the sample up to 24hours. Low temperature is known to slow down glycolysis ex-vivo. The significant reduction of the glucose level when samples were delayed beyond 24 hours may not be unconnected with glycolysis ex-vivo and the fluctuation in the room temperature over and above the course of the 24 hours. This position appears to be agreed by Mingoas et al. [23].

5. CONCLUSION

In conclusion, where possible, it is recommended that the established best practices in processing and analyzing samples be adhered to strictly. Where it is not possible, based on the results of this work, reasonably stable results for blood glucose determination could be obtained within 24 hours especially in areas of low atmospheric temperatures and no sample should be used for analysis when delayed beyond 24 hours as there would be gross reduction of the glucose level. Therefore, instead of discarding the sample, it might seem more appropriate that the sample be analyzed and the result reported indicating the number of hours delayed.

6. LIMITATIONS OF THE STUDY

The average lower atmospheric temperature of the study location (Jos Plateau State, Nigeria) as compared to other states may have influence on the result of this study.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

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

ACKNOWLEDGEMENT

The participation of the 300 level students (2022 class) of Medical Laboratory Technician Program students of Federal School of Medical Laboratory Technology Jos in this study is highly acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bishop ML, Fordy PE, Schoeff EL. Clinical chemistry techniques, principles, correlations. 6th (ed). Lippincott Williams & Wilkins. 351 West Camden Street Baltimore. 2010:309-327.
2. Peoples gazette. Skilled health worker migration is a lesson Nigeria must learn. Available:https://gazettengr.com/anna-suberu-skilled-health-worker-migration-is-a-lesson-nigeria-must-learn/. Access on 13 February 2023
3. The guardian. Equip Nigerian hospitals like UK's. Available:https://guardian.ng/opinion/equip-nigerian-hospitals-like-uks/. Access on 13 February 2023
4. Adeleye JO. The hazardous terrain of diabetes mellitus in Nigeria: The time for action is now. Research Journal of Health Sciences. 2021;9(1):69-75. Available:https://doi.org/10.4314/rejhs.v9i1.8
5. Kavitha A, Suresh PJ. Evaluation of glucose oxidase and hexokinase methods. International Journal of Biotechnology & Biochemistry. 2018;14(1):51–58.
6. Ibanga IE, Etukudoh NS, Olumide OB, Uchejeso OM, Nwapi LL, Bwotle FY. Hypoglycemic and hypolipidemic effect of bitter kola (*Garcinia kola*) seed extract on alloxan-induced diabetic albino rats. Journal of Biosciences and Medicines. 2020;8:127-134. Available:https://doi.org/10.4236/jbm.2020.86012
7. Etukudoh NS, Uchejeso OM, Etim II. Bitter kola (*Garcinia kola*) as herbal remedy for diabetes. Current Topics in Medicine and Medical Research. 2021;12:61-64. DOI: 10.9734/bpi/ctmamr/v12/6013D
8. Imperatore G, Mayer-Davis EJ, Orchard TJ, Zhong VW. Prevalence and incidence of type 1 diabetes among children and adults in the United States and comparison with non-U.S. countries. In: Cowie CC, Casagrande SS, Menke A, et al., eds.

Diabetes in America, 3rd ed. Bethesda, MD: National Institutes of Health. 2017:2-20.

9. Passey RB, Gillum LR, Fuller JB. Evaluation and comparison of 10 glucose methods and the reference method recommended in the proposed product class standard. Clinical Chemistry. 1977;23(1):131–139.
Available:https://doi.org/10.1093/clinchem/23.1.131
10. Higgins C. Measurement of circulating glucose: The problem of inconsistent sample and methodology; 2008.
Available; http://acutecaretesting.org
Accessed 14 February 2023
11. Stahl M, Jørgensen LG, Hyltoft PP, Brandslund I, Olivarius N, Borch-Johnsen K. Optimization of preanalytical conditions and analysis of plasma glucose. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. Scandinavian Journal Clinical Laboratory Investigation. 2001;61:169-79.
Available:https://doi.org/10.1080/00365510 1300133612
12. World Health Organization (WHO). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. Geneva: World Health Org. 2006:1-41.
ISBN 978 92 4 159493 6
13. Sacks DB, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care. 2011;34(6):e61–e99.
DOI: 10.2337/dc11-9998
14. Uchida K, Matuse R, Toyoda E, Okuda S, Tomita S. A new method of inhibiting glycolysis in blood samples. Clinica Chimica Acta. 1988;172:101-8.
Available:https://doi.org/10.1016/0009-8981(88)90125-8
15. Flynn DN, Carroll C, Farren M, McKeating A, Turner MJ. Impact of implementing pre-analytical laboratory standards on the diagnosis of gestational diabetes mellitus: A prospective observational study. Clinical Chemistry. 2016;62:387-91.
Available:https://doi.org/10.1373/clinchem.2015.247478
16. Nwosu OK, Nwani CD. Stability of serum/plasma glucose for the diagnosis of diabetes mellitus. Bio-Research. 2008;6(1):380–383.
Available:https://doi.org/10.4314/br.v6i2.28671
17. Weatherspark.com. August Weather in Jos Nigeria.
Available:https://weatherspark.com/m/58551/8/Average-Weather-in-August-in-Jos-Nigeria.
Access on 15 February 2023
18. Randox Laboratories Ltd. Glucose (GLUC-PAP); 2022.
Available:www.randox.com
19. Dharmasena IA, Deepani S, Anoja PA. Effect of sample volume variation and delay in analysis on plasma glucose concentration in Sri Lankan healthy adults. Hindawi Scientifica. 2021:1-5.
Available:https://doi.org/10.1155/2021/6061206
20. Chan AYW, Cockramj CS, Swaminathan R. Effect of delay in separating plasma for glucose measurement upon the interpretation of oral glucose tolerance tests. Ann Clin Biochem. 1990;27:73-74.
21. Chand B, Deepak S, Poudel SK. Effect of delayed serum separation on glucose concentration of dogs at room temperature. Advances in Animal and Veterinary Science. 2020;8(8):800-803.
Available:http://dx.doi.org/10.17582/journal.aavs/2020/8.8.800.803
22. Jangam SR, Gary H, Timothy CD. Impact of glucose measurement processing delays on clinical accuracy and relevance. Journal of Diabetes Science and Technology. 2013;7(3):660–668.
23. Mingoas JPK, Somnjom DE, Moffo F, Mfopit YM, Awah-Ndukum J. Effect of duration and storage temperature on haematological and biochemical parameters of clinically healthy Gudali Zebu in Ngaoundere, Cameroon. Singapore Journal of Scientific Research. 2020;10(2):190-196.
DOI:10.3923/sjsres.2020.190.196

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97077