ijlpr 2023; doi 10.22376/ijlpr.2023.13.1.L136-142

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Biochemistry

Can Serum Replace Plasma for Glucose Estimation? – An Integrity Check

Vasanthan. M.[1]* and Vinodhini. V. M[1]

[1]*Department of Biochemistry, SRM Medical College Hospital & Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, TN, India.*

Abstract: Glucose is the most common parameter to be estimated in a clinical laboratory. However, being highly unstable, the reported values often lead to under diagnosis of diabetes mellitus. This exerts a greater pressure on the laboratories as this reflects their quality. The aim of the study was to analyse the stability of the parameter in serum and plasma samples in a time delay of 1 hour and 2 hours in a tertiary care laboratory in South-India. The study included 125 samples of serum and plasma. Glucose was estimated by Hexokinase method. The serum and plasma samples were separated following the standard operating procedures of the laboratory. They were grouped as A and B. The samples of Group A and B were estimated for glucose concentration at 0, 1 and 2 hours and the values were noted against the time. The serum samples were stored at 2-4 degrees Celsius in between the estimations at 1 and 2 hours. The glucose concentration at 0, 1 and 2 hours were compared to each other with the help of Analysis of variance (ANOVA). The significance p of the ANOVA test performed to compare serum glucose concentrations analyzed at 0, 1 and 2 hours was found to be 1.000, with a p-value of 0.999 for the plasma sample glucose concentrations**.** It was evident that glucose concentration was stable with the p value closer to 1.000 in plasma samples estimated at 0, 1 and 2 hours. Plasma can be replaced with serum during the first hour without much alterations in the glucose concentration when stored at 2-4 degrees Celsius.

Keywords: Glucose, Plasma, Serum, Stability and Storage.

***Corresponding Author**

Vasanthan. M , Department of Biochemistry, SRM Medical College Hospital & Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, TN, India.

Received On 24 August, 2022
Revised On 11 November, 2022
Accepted On 24 November, 2022
Published On 2 January, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Vasanthan. M and Vinodhini. V. M , Can Serum Replace Plasma for Glucose Estimation? – An Integrity Check.(2023).Int. J. Life Sci. Pharma Res.13(1), L136-142 http://dx.doi.org/10.22376/ijlpr.2023.13.1.L136-142

1. INTRODUCTION

A quality clinical laboratory aims at accuracy and precision of parameters. One of the most common parameters run in a clinical laboratory is blood glucose. The measurement of blood glucose determines the diagnosis and management of diabetes[1]. The diagnostic criterion for diabetes according to the World Health Organization (WHO) is ≥ 126 mg/dl in fasting and ≥ 200 mg/dl in post-prandial state[2]. Both hypoglycaemia and impaired fasting glucose should be considered as predictors of stroke and coronary artery disease risk, reports a study[3]. This insists the importance of accurate estimation of glucose in clinical laboratories. The major issue being the errors at various phases of sample processing. Pre-analytical phase contributes to the maximum of all errors in different phases of the laboratory testing. Pre-analytical phase includes collection of samples, transportation to lab, and separation. In some labs, the samples are stored for a period of time and processed together. This in turn may lead to instability of the estimated parameters[4]. Therefore, all laboratory operations should be based on Standard operating procedure (SOP). About two third of the clinicians' view on approach to a patient's treatment are based upon laboratory results[5]. Providing quality test results depends not only on proper sample analysis and reporting, but also on sample integrity prior to analysis[6]. The concentration of glucose keeps decreasing till it reaches 7% due to glycolysis in various blood cells like erythrocytes[7-9]. This rate of fall depends on various factors like concentration of glucose, white blood cells and mainly the processing condition of the laboratory[2]. According to the study, only a time delay in processing serum samples for glucose led to underestimation of diagnosing hyperglycaemic state. Each laboratory has varying environments based on the temperature maintained, water used for analysis, the technical personnel involved in the laboratory and the routine procedure. The principle of glucose estimation would also vary. According to a few studies, serum glucose is not suggested for glucose estimation[10,11]. In contrast, Kang et al. compared serum glucose and plasma glucose for a longer duration of one month[12]. The significance of glucose stability was evident from a study conducted in Nigeria. The study examined changes in glucose concentration over 3 days in serum and plasma samples stored at room temperature and at 4 degrees Celsius. According to the study, recording the length of time between sample collection and analysis, rational adjustments can be made for declining glucose concentrations over time[13]. This was in concordance to the study conducted by Pant V et al according to which, Serum glucose measurements are acceptable if serum separation by centrifugation is achieved within 30 minutes and the advantage of using a serum tube is avoiding additional blood collection in another Sodium fluoride tube and reducing turn around time[14]. A delay in sample processing and different principles of the estimation of the parameter may result in misdiagnosis of diabetes mellitus, particularly closer to the diagnostic criteria. Hence, the study was aimed to check the integrity of the serum and plasma samples for glucose at different time intervals. The data obtained will be helpful to define acceptable time delay in processing plasma samples for glucose in serum and also to use serum glucose as an alternative to plasma samples. This outcome of the study shall enable the avoidance of asking repeat samples, taking more volumes of blood and also shortens the turnaround time.

2. MATERIALS AND METHODS

2.1 Inclusion Criteria

Each group with 125 serum and 125 plasma samples of the patients of age group 20-50 were included in the study.

2.2 Exclusion Criteria

Haemolysed, icteric, lipemic and samples of the age group less than 20 and greater than 50 were excluded from the study.

2.3 Collection and Analysis of Samples

The Institutional Ethical Committee (IEC) was obtained before the initiation of the study from the institution (IEC No:1555/IEC/2019). The study was performed in a tertiary care hospital and samples were collected from those who attended the outpatient and inpatient departments. Glucose was estimated in the collected serum and plasma from the same participants' samples at 0, 1 and 2 hours. The study was conducted in a laboratory belonging to tertiary care health care centres in South-India. The study involved serum and plasma samples estimated for glucose at 0, 1 and 2 hours. The sample was processed at the Department of Biochemistry at the Central clinical laboratory of the institution. Blood samples of around 3 mL were collected as per the SOP followed in the phlebotomy. The collected samples were labelled and was transported to the Biochemistry department within 10 minutes. Precautions were strictly followed in sample transportation to avoid haemolysis. The received samples were allowed to clot for 20-30 minutes. The samples were centrifuged at 3500 rpm for 12 minutes. The standard pre-analytical procedure was followed commonly in both the laboratories. Serum was separated and loaded in the auto-analyser, Beckman-Coulter AU480 and glucose was estimated by Hexokinase method at 0, 1 and 2 hours. Plasma samples of the same patients were analysed for glucose at 0, 1 and 2 hours. The samples between the runs were stored at 2-8 degrees Celsius. *Statistical analysis:* Means and Standard deviations of Glucose between the serum and plasma samples (n=125) were analysed with the help of One-way Analysis of variance (ANOVA) and the significance within the group was analysed with the help of Post HOC – Tukey's HSD test. The statistical workout was carried out with the help of *SPSS software version 16.0.*

3. RESULTS

The separated plasma and serum samples were analysed for glucose at 0 hr, 1 hr and 2 hrs. The values obtained were expressed in means and standard deviations. The means and standard deviations were compared between serum and plasma at different time intervals. Furthermore, the values were correlated between different time intervals within the group.

Table 1: Comparing glucose levels in plasma and serum at 0 hr, 1 hr and 2 hrs. (one-way ANOVA)

Parameters (n=150)	0 hour(Group A)	1 hour(Group B)	2 hours (Group C)	p Value
Plasma	134.1 ± 44.1	133.7 ± 43.8	133.4 ± 44.4	**.999**
Serum	130.8 ± 43.9	130.5 ± 43.7	130.5 ± 43.5	**1.000**

Values are Mean ± Standard deviation. N=150

Table 1 shows the Means and Standard deviations of the serum and plasma samples analysed for glucose concentration at 0 (Group A), 1 (Group B) and 2 hours (Group C) using One-way ANOVA. Glucose concentration was estimated by the Hexokinase method in both serum and plasma samples. The serum samples were stored at temperature of 2-8 degrees Celsius, thawed and then run for glucose in the fully automated analyser Beckman-Coulter AU480.

Table 2: Post HOC – Tukey's HSD test between the groups in plasma sample

Group I	Plasma glucose	p value
0 hour	1 hour	1.000
	2 hours	.999
1 hour	0 hour	1.000
	2 hours	1.000
2 hours	0 hour	.999
	1 hour	1.000

Post-HOC – Tukey's test was conducted to identify the significance of the plasma samples (Group I) run at 0 hr, 1 hr and 2 hrs. Significance of glucose concentration in plasma samples at 0 hour with 1 hour and 2 hours, 1hour plasma glucose concentration with 0 hour and 2 hours, and 2 hours' plasma glucose concentration with 0 hour and 1 hour were calculated.

Table 3: Post HOC – Tukey's HSD test between the groups in serum sample

Group II	Serum glucose	p value
0 hour	1 hour	1.000
	2 hours	1.000
1 hour	0 hour	1.000
	2 hours	1.000
2 hours	0 hour	1.000
	1 hour	1.000

Post-HOC – Tukey's test was conducted to identify the significance of the serum samples (Group II) run at 0 hr, 1 hr and 2 hrs. Significance of glucose concentration in serum sample at 0 hour with 1 hour and 2 hours, 1 hour serum glucose concentration with 0 hour and 2 hours, and 2 hours serum glucose concentration with 0 hour and 1 hour were calculated.

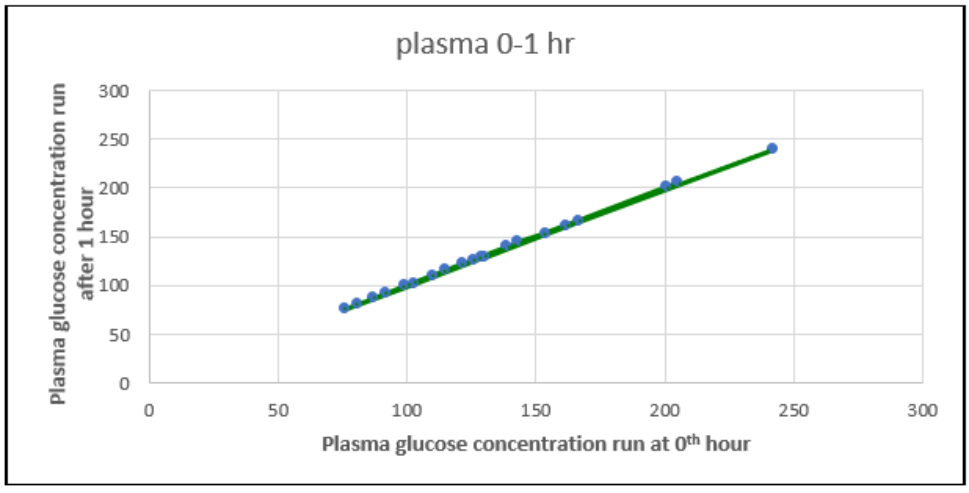

Fig 1: Correlation between plasma glucose concentration runs at 0 hour and 1 hour

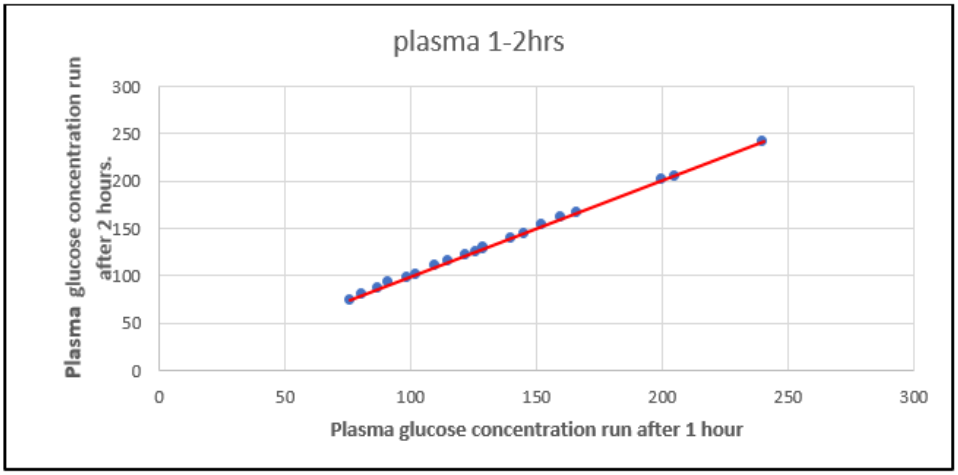

Fig 2: Correlation between plasma glucose concentration runs at 1 hour and 2 hours.

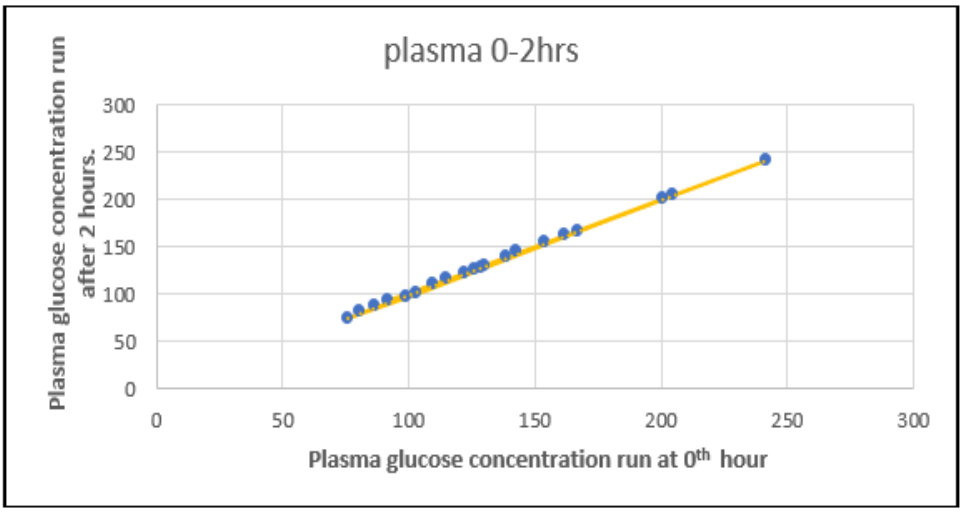

Fig 3: Correlation between plasma glucose concentration runs at 0 hour and 2 hours.

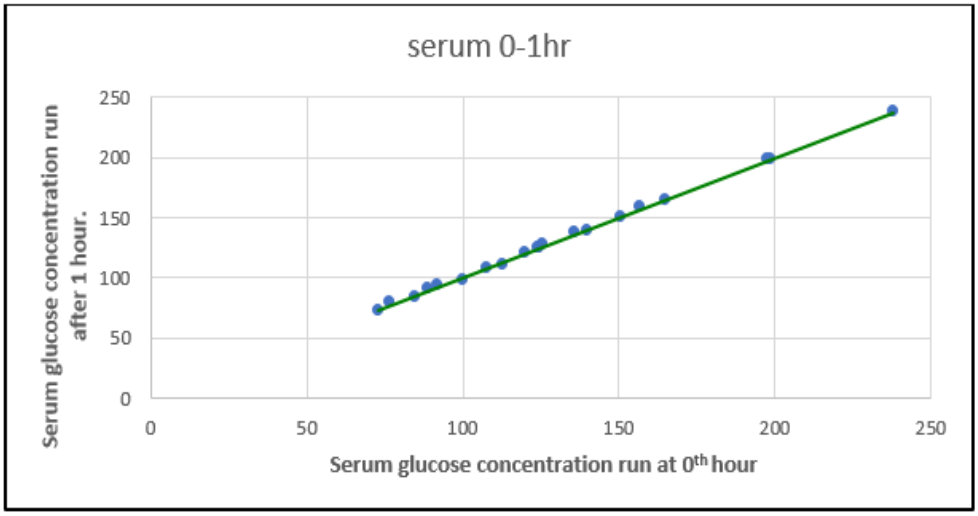

Fig 4: Correlation between serum glucose concentration run at 0 hour and 1 hour.

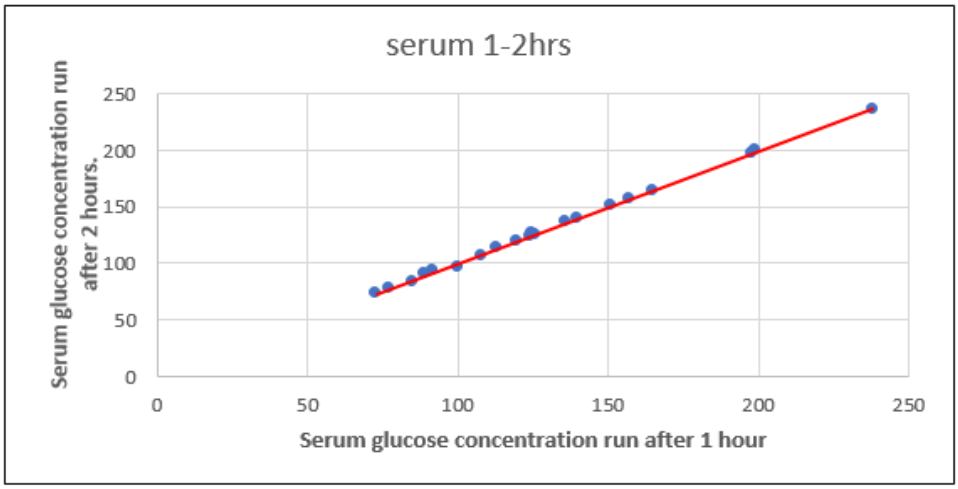

Fig 5: Correlation between serum glucose concentration run at 1 hour and 2 hours.

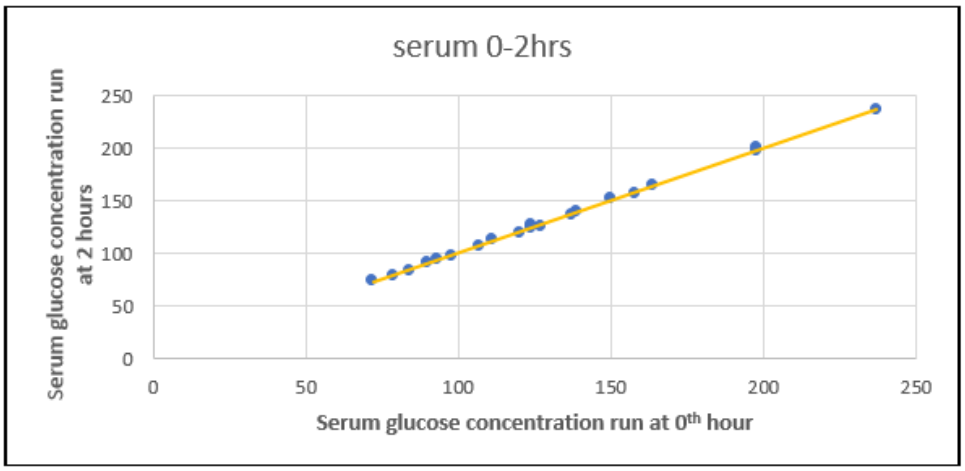

Fig 6: Correlation between serum glucose concentration runs at 0 hour and 2 hours.

4. DISCUSSION

According to the ISO 15197, 95 % of the estimated glucose should fall within 20% of glucose concentration greater than 75 mg/dl[15]. This is in concordance with the ADA guidelines of measured blood glucose inaccuracy lesser than 5%[16]. Ginsberg BH studied various factors influencing blood glucose concentration like haematocrit and even hand hygiene[17]. The mean concentration of the serum glucose at 0, 1 and 2 hours were 130.8 ± 43.9, 130.5 ± 43.7 and 130.5 ± 43.5 mg/dl respectively. The mean concentration of the plasma glucose at 0, 1 and 2 hours were 134.1 ± 44.1, 133.7 ± 43.8 and 133.4 ± 44.4 mg/dl respectively. The significance p value was found to be .999 using ANOVA for plasma and 1.000 for serum. Post HOC – Tukey's HSD (honestly significant difference) test was done to compare the glucose concentration between the groups (0, 1 and 2 hours) in serum and plasma samples. Plasma samples should be separated as soon as possible after centrifugation to avoid glycolysis, reports a study by Kim HS[18]. This was in contrast to the current study. In case of proper handling, serum samples can be a better sample than plasma for estimation of glucose concentration, reports a study conducted by Frank EA[19]. This finding was in concordance to the current study. The Tukey's HSD test showed a p value between .999 to 1.000 among plasma samples and a p value of 1.000 among serum samples in the current study. Glucose concentration is reduced by glycolysis by red blood cells, white blood cells, and platelets, breaking down glucose at a rate of 5% to 7% per hour[20]. The above finding maybe when the temperature was not maintained and the sample therefore had lost the integrity. Changes in temperature can affect blood glucose readings in other ways as well[21]. This was in concordance to the current study as in the study, there was a stringent temperature control throughout the study. The samples were immediately shifted to storage post-analysis. Red blood cells are unique among body cells in that they contain a significant amount of glucose within the cell, which is responsible for the influence of haematocrit on blood glucose concentration[22]. The current study did not involve any haematocrit parameters. The above results show minimal changes in plasma glucose with no significant decrease in serum glucose concentration after 1 hour and 2 hours. This may be explained by the storage of serum samples at 2-8 degrees Celsius in between the estimation of glucose concentration. Serum samples held at 4 degrees C should be analysed within 72 hours following blood collection for blood glucose determination, while samples stored at 32 degrees C should be analysed within 2 hours[23]. This was in concordance to the current study. After two hours at room temperature, centrifuged samples of salivary glucose can be accurately estimated, reported a study[24]. The above-mentioned study was carried out in saliva as sample but the finding was related to the current study in sample integrity. Another similar study concluded that, Glucose concentration decreased as serum clot/plasma clot contact time increased[25]. This was consistent with current research.

5. CONCLUSION

When maintained at a lower temperature of 2-8 degrees Celsius, glucose can be estimated in serum without much of a decrease in concentration and can be used as an alternative sample for plasma in the first few hours of sample collection. The study outcome shall be of greater importance in utilising the serum sample for glucose estimation when there is no time delay. Moreover, every laboratory should perform an integrity check of the samples stored and processed in their laboratories, according to their standard operating procedure to ensure timely and quality reporting.

6. AUTHORS CONTRIBUTIONS STATEMENT

Vasanthan M conceived of the presented idea, collected the samples and performed the analytical methods. Vinodhini VM verified the analytical methods and supervised the findings of this work. All the authors discussed the results and contributed to the final manuscript.

7. ACKNOWLEDGMENT

We sincerely thank the volunteers who participated in our study.

8. CONFLICT OF INTEREST

Conflict of interest is declared none.

9. ETHICAL CLEARANCE

Clearance was obtained for the study from the Institutional Ethical Committee (No:1555/IEC/2019). The study participants involved themselves voluntarily in the study after obtaining a written consent.

10. REFERENCES

1. Schrot RJ, Patel KT, Foulis P. Evaluation of inaccuracies in the measurement of glycemia in the laboratory, by glucose meters, and through measurement of hemoglobin A1c. Clin Diabetes. 2007;25(2):43-9. doi: 10.2337/diaclin.25.2.43.
2. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem. 2002;48(3):436-72. doi: 10.1093/clinchem/48.3.436, PMID 11861436.
3. Park C, Guallar E, Linton JA, Lee DC, Jang Y, Son DK et al. Fasting glucose level and the risk of incident atherosclerotic cardiovascular diseases. Diabetes Care. 2013 Jul;36(7):1988-93. doi: 10.2337/dc12-1577, PMID 23404299.
4. Quality of diagnostic samples. Recommendations of the working group on preanalytical quality of the German Society for Clinical chemistry and laboratory medicine. Vol. 2. WHO/DIL/LAB.99.1 Rev; 2009. p. 85.
5. Forsman RW. Why is the laboratory an afterthought for managed care organizations? Clin Chem. 1996;42(5):813-16. doi: 10.1093/clinchem/42.5.813, PMID 8653920.
6. Funk DA. Sample integrity and preanalytical variables. Quality in laboratory hemostasis and thrombosis. 2nd ed; 2013. p. 45-56.
7. Overfield CV, Savory J, Heintges MG. Glycolysis: A re-evaluation of the effect on blood glucose. Clin Chim Acta. 1972;39(1):35-40. doi: 10.1016/0009-8981(72)90296-3, PMID 5038762.
8. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem. 1989;35(2):315-7. doi: 10.1093/clinchem/35.2.315, PMID 2914384.
9. Gambino R. Glucose: A simple molecule that is not simple to quantify. Clin Chem. 2007;53(12):2040-1. doi: 10.1373/clinchem.2007.094466, PMID 18267929.
10. American Diabetes Association. Standards of medical care in diabetes–2010. Diabetes Care. 2010;33(Suppl 1);Suppl 1:S11-61. doi: 10.2337/dc10-S011, PMID 20042772.
11. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva: WHO; 2006.
12. Kang JG, Park CY, Ihm SH, Park SW. A potential issue with screening prediabetes or diabetes using serum glucose: a delay in diagnosis. Diabetes Metab J. 2016;40(5):414-7. doi: 10.4093/dmj.2016.40.5.414, PMID 27766249.
13. Nwani C, OK N. Stability of serum/plasma glucose for the diagnosis of diabetes mellitus. Biol Res. 2008;6(2):380-3.
14. Pant V, Gautam K, Pradhan S, Pyakurel D, Shrestha A. Blood glucose concentration measured in EDTA/F plasma and serum in a referral clinical laboratory in Nepal. J Pathol Nep. 2021;11(1):1837-41. doi: 10.3126/jpn.v10i2.32351.
15. International Organization for Standardization. In vitro diagnostic test systems. Requirements for blood-glucose monitoring system for self-testing in managing diabetes mellitus. Geneva: International Organization for Standardization; 2003. Reference number ISO 15197:2003 (E).
16. American Diabetes Association. Standards of medical care in diabetes—2008. Diabetes Care. 2008;31;Suppl 1:S12-54. doi: 10.2337/dc08-S012, PMID 18165335.
17. Ginsberg BH. Factors affecting blood glucose monitoring: sources of errors in measurement. J Diabetes Sci Technol. 2009 Jul 1;3(4):903-13. doi: 10.1177/193229680900300438, PMID 20144340.
18. Kim HS. Blood glucose measurement: is serum equal to plasma? Diabetes Metab J. 2016 Oct;40(5):365-6. doi: 10.4093/dmj.2016.40.5.365, PMID 27766243.
19. Frank EA, Shubha MC, D'Souza CJ. Blood glucose determination: plasma or serum? J Clin Lab Anal. 2012 Sep;26(5):317-20. doi: 10.1002/jcla.21524, PMID 22585749.
20. Haupt A, Berg B, Paschen P, Dreyer M, Häring HU, Smedegaard J et al. The effects of skin temperature and testing site on blood glucose measurements taken by a modern blood glucose monitoring device. Diabetes Technol Ther. 2005;7(4):597-601. doi: 10.1089/dia.2005.7.597, PMID 16120031.
21. Somogyi MJ. The distribution of sugar in blood. Biol Chem. 1928:117-27.
22. Barreau PB, Buttery JE. Effect of hematocrit concentration on blood glucose value determined on

glucometer II. Diabetes Care. 1988;11(2):116-8. doi: 10.2337/diacare.11.2.116, PMID 3383731.
23. Nwosu OK, Nwani CD. Stability of serum/plasma glucose for the diagnosis of diabetes. Mellit Bio Res. 2008;6:380-3.
24. Vasupradha G, Sankar K, Rajendran R, Nitya K, Murugaboopathy V, Pallavan B. Effect of 2 h of room temperature storage on salivary glucose concentration. J Oral Maxillofac Pathol. 2021 Sep-Dec;25(3):446-8. doi: 10.4103/jomfp.jomfp_71_21, PMID 35281178.
25. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem. 2002;48(12):2242-7. doi: 10.1093/clinchem/48.12.2242, PMID 12446483.