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Original Research Article

Study of glucose specimen integrity in various blood collection tubes over a period of 24 hours

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ABSTRACT

Background: Blood glucose testing is performed to diagnose and monitor conditions such as diabetes mellitus and gestational diabetes in pregnant women. Accurate measurement of plasma glucose concentration is an important component. Here, we have compared the glucose estimation in specimen collected in various collection tubes.
Methods: The samples were stored at 2-4°C temperature and at 0, 4, 12 and 24 hrs aliquots were taken and were processed. Plasma glucose was determined using the glucose oxidase enzymatic methods on the Beckman coulter AU700. All the results at 0 hour were compared with results at 4 hours, 12 hours and 24 hours. Also results of fluoride tubes were compared with results of EDTA, heparin and plane tubes.
Results: We have found no significant difference between results of glucose estimation obtained using samples collected in serum tubes, EDTA tubes, fluoride tube and lithium heparin tube even after 24 hours after separation of serum/plasma.
Conclusions: From this study we have concluded that blood samples collected in plane serum tube, fluoride tubes, EDTA tubes and heparin tubes produce similar results for estimation of glucose if the collected samples are centrifuged immediately after collection for separation of plasma and serum.

Keywords: Analysis, Anticoagulants, Glucose, Preservatives, Stability, Storage

INTRODUCTION

Blood glucose estimation is one of the commonest investigations performed in medical laboratory. The blood glucose estimation is performed for understanding the glycemic status of our body. Blood glucose testing is performed to diagnose and monitor conditions such as diabetes mellitus and gestational diabetes in pregnant women. Accurate measurement of plasma glucose concentration is an important component

Accurate measurement of blood glucose levels using standardized preanalytical procedures is essential for defining gestational diabetes mellitus, because increases in blood glucose even within the non-diabetic range.[1] Evers IM, et al has found that risk of complications of pregnancy in women with type 1 diabetes to be associated with perinatal complications.[2] The American Diabetes Association guidelines recommend that glucose concentrations be measured in plasma samples separated from cells within 60 minutes of collection.[3] However, in practice, rapid sample processing is not always feasible. In particular, the oral glucose tolerance test (most commonly used for screening and diagnosis of gestational diabetes) poses challenges for sample processing, as it requires sample collection over the span of one to three hours. In settings where immediate sample processing is not achievable, guidelines recommend the use of glycolysis inhibitors such as sodium fluoride (NaF).[4]

Here, we have compared the glucose estimation in specimen collected in various collection tubes. We have

collected the samples in lithium heparin tubes, plane tubes, fluoride tubes, EDTA tubes. Glucose estimations were done over a period of time stored in appropriate conditions and results were compared.

Rationale of the study

Estimation of blood glucose levels is a critical component in the diagnosis and follow-up of diseases related to the metabolic disorder diabetes mellitus and its variant diabetes in pregnancy. In such conditions, the availability of accurate blood sugar levels for sound medical practices is paramount. Nevertheless, factors before the analysis such as the kind of blood collection tube and the duration between blood collection and analysis have been shown to affect the glucose levels in blood.

Taking into consideration the challenges in the logistics of sample processing, particularly in developing countries or during long protocols such as the oral glucose tolerance test, it is critical to study the time stability of glucose in various tubes. Common practice would recommend the use of sodium fluoride and ammonium oxalate which are glycolysis inhibitors to assist the preservation of glucose levels. However, their use in clinical laboratories is still limited by their price and availability.

The following gaps will be addressed by this study which endeavors to investigate tube types and the 24-hour stability of glucose stored in whole blood. This will be achieved by studying the differences between these tubes in results obtained with difference time intervals, with difference storage temperature 2 and 4 degrees (standard conditions). This will help in establishing whether there are alternative tubes which can give dependable results and thus extend the range of practices accepted for glucose testing in various health care provision settings.

The findings will guide the clinician or laboratory personnel whether or not combined plasma or serum processing or appropriate storage will require refraining from using certain glycolysis inhibitors like fluoride providing efficiency and cost effectiveness without losing diagnostic quality. This finding is very important in enhancing lab operations and the availability of quality glucose testing services to the world.

METHODS

Sample population

Human venous blood samples were collected from 45 volunteers. The subjects were fully informed of the protocol and written consent was obtained according to the Helsinki Declaration.

Study design

The study employs a prospective, comparative experimental design with the following characteristics:

Study place and period

The study was conducted at Meenakshi Labs, Madurai from December 2023 to March 2024. The study duration was 4 months

Population and sampling

Sample size

Blood samples were collected from 45 healthy volunteers.

Sampling method

Convenience sampling of consenting individuals.

Intervention/procedure

Blood from each participant was divided into four different tubes with varying preservatives/anticoagulants: K_2EDTA tubes, sodium fluoride (NaF/Na_2EDTA) tubes, lithium heparin tubes, plane serum tubes.

After immediate centrifugation, serum or plasma was separated and aliquoted for storage.

Storage conditions

Separated serum/plasma was stored at a controlled temperature (2-4°C).

The samples were stored at 2-4°C temperature and at 0-, 4-, 12- and 24-hours aliquots were taken and were processed. Plasma glucose was determined using the hexokinase enzymatic methods on the Beckman coulter AU700. Strict internal quality control was maintained during analysis of all the samples.

All the results at 0 hour were compared with results at 4 hours, 12 hours and 24 hours. Also results of fluoride tubes were compared with results of EDTA, heparin and plane tubes.

Time-dependent analysis

Glucose levels were measured at four specific time points: 0-, 4-, 12-, and 24-hours post-collection.

Statistical analysis

Data were analyzed using the two-tailed paired Student's t-test to compare glucose levels at different time intervals and among different tube types. A p value <0.05 was considered statistically significant.

This design allows the evaluation of glucose stability in different blood collection tubes over time, addressing both practical and clinical implications for pre-analytical sample handling.

RESULTS

Results obtained by running glucose tests in samples collected in various tubes at different times lines are shown in Tables 1-4.

Results obtained by running glucoses test in different tubes immediately after collection in different tubes are shown in Table 5.

Table 1: Glucose testing over period of 24 hours for sample collected in EDTA tube.

Samples collected in EDTA tube			
0 hours versus 4 hours	**0 hours versus 8 hours**	**0 hours versus 12 hours**	**0 hours versus 24 hours**
t-value is 0.28	t-value is 0.36	t-value is 0.39	t-value is 0.54
P value is 0.77	P value is 0.71	P value is 0.69	P value is 0.58

Table 2: Glucose testing over period of 24 hours for sample collected in plane tube.

Samples collected in serum tube			
0 hours versus 4 hours	**0 hours versus 8 hours**	**0 hours versus 12 hours**	**0 hours versus 24 hours**
t-value is 0.28	t-value is 0.33	t-value is 0.39	t-value is 0.45
P value is 0.77	P value is 0.73	P value is 0.69	P value is 0.65

Table 3: Glucose testing over period of 24 hours for sample collected in fluoride tube.

Samples collected in fluoride tube			
0 hours versus 4 hours	**0 hours versus 8 hours**	**0 hours versus 12 hours**	**0 hours versus 24 hours**
t-value is 0.22	t-value is 0.33	t-value is 0.34	t-value is 0.37
P value is 0.82	P value is 0.73	P value is 0.72	P value is 0.71

Table 4: Glucose testing over period of 24 hours for sample collected in lithium heparin tube.

Samples collected in lithium heparin tube			
0 hours versus 4 hours	**0 hours versus 8 hours**	**0 hours versus 12 hours**	**0 hours versus 24 hours**
t-value is 0.34	t-value is 0.38	t-value is 0.40	t-value is 0.64
P value is 0.72	P value is 0.70	P value is 0.69	P value is 0.51

Table 5: Glucose testing results compared with sample collected in fluoride tube with sample collected in plane serum tube, lithium heparin tube and EDTA tube.

Fluoride versus serum at 0 hours	Fluoride versus heparin at 0 hours	Fluoride versus EDTA at 0 hours
t-value is 0.07	t-value is -0.04	t-value is 0.03
P value is 0.93	P value is 0.96	P value is 0.97

Stability across tube types

There was no significant difference in glucose estimation results among the four types of tubes when serum/plasma was separated immediately after sample collection.

This consistency was maintained even after storage for up to 24 hours at 2-4°C.

Time-dependent analysis

Glucose levels measured at 4, 12, and 24 hours were statistically comparable to the baseline (0-hour) levels in all tube types.

Results showed negligible degradation or variation, provided that the serum/plasma was promptly separated and stored appropriately.

Comparative performance

Glucose levels in fluoride tubes were not significantly different from those in EDTA, lithium heparin, or plane serum tubes across all time points.

Statistical analysis showed p values >0.05 for all comparisons, confirming the absence of significant discrepancies.

DISCUSSION

It was seen from this study that if there was no significant difference between glucose estimation even if the samples are collected in EDTA tubes or lithium heparin tubes or plane tubes or fluoride tubes provided that immediately after collection the samples must be centrifuged to separate the plasma or serum. Also the plasma/serum separated when was stored at 2-8°C and glucose estimation was done at 4 hours, 8 hours, 12 hours and 24 hours the results were not significantly different from the results which are obtained at 0 hours after sample collection.

Roccaforte et al have also demonstrated that no statistically significant differences were found in blood samples which were centrifuged within 30 minutes collected in plane serum and lithium-heparin plasma.[5]

Pant et al have concluded in their study that the difference between plasma glucose at 30 minutes and serum glucose at 4 hours was only 1.9% which is not clinically significant.[6]

Dimeski et al also report that at 4 hours, the glucose concentration in serum and heparin compared with NaF/KOx tubes was statistically insignificant from one another results showed that serum and NaKOx were very similar at 4 hours.[7] Fernandez et al showed that barrier serum tubes (SST) and NaF/potassium oxalate (NaF/KOx) plasma tubes can be used under survey collection and processing conditions to measure glucose with no difference in reported results.[8]

Kevin et al has shown that there is no difference between use of a fluoride tube or a serum separator tube for up to 24 hours.[9]

We have found no significant difference between results of glucose estimation obtained using samples collected in serum tubes, EDTA tubes, fluoride tube and lithium heparin tube even after 24 hrs after separation of serum/plasma.[10,11] Here the important aspect to note is that the serum/plasma must be separated immediately after collection of samples.[12,13] If the plasma/serum is not separated immediately it may result into accelerated glycolysis which may cause significant difference in the results with samples collected in different anticoagulant or plane serum tube in comparison to the samples which are collected in tubes containing anti glycolytic agent such as sodium fluoride.[14,15] Storing of such samples for 24 hours may further cause increase in glycolysis resulting into deterioration of glucose results.

Limitations of the study are: sample size- the study included only 45 volunteers, which may limit the generalizability of the results to larger and more diverse populations. Healthy volunteer bias- the participants were healthy individuals, and the results may not be directly applicable to patients with abnormal glucose metabolism (e.g., diabetes mellitus or gestational diabetes). Single storage condition- samples were stored only at 2-4°C, and the study did not evaluate the effects of other storage conditions (e.g., room temperature or freezing) on glucose stability. Limited duration- the study assessed glucose stability over a maximum of 24 hours. Longer durations of storage were not considered, which could be relevant in specific scenarios such as delayed processing in remote healthcare settings. Single analytical method- glucose concentrations were measured using the glucose oxidase enzymatic method on one specific analyzer (Beckman Coulter AU700). The findings may not be generalizable to other methods or equipment. Pre-analytical variations- while the study controlled for immediate centrifugation, real-world scenarios may involve delays in processing, which could introduce variability not accounted for in this study. Focus on tube types- the study primarily compared the impact of different blood collection tubes on glucose stability.[16] It did not explore other pre-analytical factors like the impact of hemolysis, different anticoagulant concentrations, or tube material.

CONCLUSION

From this study we have concluded that blood samples collected in plane serum tube, fluoride tubes, EDTA tubes and heparin tubes produce similar results for estimation of glucose if the collected samples are centrifuged immediately after collection for separation of plasma and serum. This separated serum/plasma also produces results which are not different significantly considering the statistics if they are stored at proper temperature for 24 hours.

Recommendations

Include a larger and more diverse cohort, including patients with diabetes. Evaluate the effects of varying storage conditions and durations. Incorporate multiple analytical platforms for cross-validation of results.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991-2002.
2. Evers IM, de Valk HW, Visser GH. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. BMJ. 2004;328(7445):915.
3. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem. 2011;57:1-47.

4. Shalini L, Silambanan S. A study on the assessment of stability of glucose concentrations in serum separator gel tubes. Int J Clin Biochem Res. 2018;5(2):334-7.
5. Roccaforte V, Daves M, Platzgummer S, Lippi G. The impact of different sample matrices in delayed measurement of glucose. Clin Biochem. 2016;49(18):1412-5.
6. Pant V, Gautam K, Pradhan S, Pyakurel D, Shrestha A. Blood glucose concentration compared in EDTA/F plasma and serum in a referral clinical laboratory in Nepal. J Pathol Nep. 2021;11:1837-41.
7. Dimeski G, Yow KS, Brown NN. What is the most suitable blood collection tube for glucose estimation? Ann Clin Biochem. 2015;52(2):270-5.
8. Fernandez L, Jee P, Klein MJ, Fischer P, Perkins SL, Brooks SP. A comparison of glucose concentration in paired specimens collected in serum separator and fluoride/potassium oxalate blood collection tubes under survey 'field' conditions. Clin Biochem. 2013;46(4-5):285-8.
9. Samuel JKR, Chandrasekaran S, Karpaghavalli VG. Comparison of sodium fluoride and serum separator tubes for blood glucose estimation- what's the best option? Int J Clin Biochem Res. 2022;9(3):204-9.
10. Coward SM, O'Neill FC, McAdam L, Reilly L, McKeeman GC. Stabilization of plasma glucose: the use of newer technology and pragmatic laboratory practice. J Appl Lab Med. 2019;3(6):1028-34.
11. Fobker M. Stability of glucose in plasma with different anticoagulants. Clin Chem Lab Med. 2014;52(7):1057-60.
12. Chauhan K P, Patel J D, Prajapati S, Trivedi A. Study of specimen stability for biochemical parameters. Int J Clin Biochem Res. 2018;5(1):158-63.
13. Shaherawala J, Mangukiya K. Study of various glycolytic inhibitors for preservation of blood glucose and clinical biochemical parameters. Int J Clin Biochem Res. 2019;6(4):535-8.
14. Laessig RH, Hassemer DJ, Westgard JO, Carey RN, Feldbruegge DH, Schwartz TH. Assessment of the Serum Separator Tube as an Intermediate Storage Device with in the Laboratory. Am J Clin Pathol. 1976;66(4):653-7.
15. Reshma DC. Determining the efficacy of citrate buffer tubes compared to Sodium fluoride tubes as inhibitor of glycolysis in glucose estimation. Int J Clin Biochem Res. 2019;6(3):299-302.
16. Juricic G, Bakliza A, Saracevic A, Kopcinovic LM, Dobrijevic S, Drmic S, et al. Glucose is stable during prolonged storage in un-centrifuged Greiner tubes with liquid citrate buffer, but not in serum and NaF/KOx tubes. Clin Chem Lab Med. 2016;54(3):411-8.

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