

STUDENT ARTICLE

Sodium Fluoride tubes versus plain tubes for In vitro blood glucose analysis

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Abstract

Background:

In terms of diagnosis and management of diabetes mellitus, the most commonly requested core biochemistry test worldwide is measurement of glucose in blood. Despite the wide use of sodium fluoride (NaF) as the specimen preservative, its use has been critically questioned.

Objective:

This study investigated the stability of glucose in blood obtained into plain tubes in comparison with sodium fluoride/potassium oxalate (NaF/K₂C₂O₄) tubes in a routine laboratory setup.

Methods:

Sixty one pairs of blood specimens were collected into plain tubes and NaF/K₂C₂O₄ tubes. Following separation of plasma (NaF/K₂C₂O₄ tubes) and serum (plain tubes), glucose concentration was measured. Five determinants were obtained; the baseline glucose value, glucose concentrations at one, two, four and six hours after collection of blood into NaF/K₂C₂O₄ or plain tubes.

Results:

A high rate of haemolysis was observed in NaF/K₂C₂O₄ tubes when compared with that of plain tubes (11% vs. 4%). No effect of tube type on serum/plasma glucose concentration (p>.05) was noted until two hours post collection. A significant reduction (p<.001) of blood glucose in plain tubes was observed in comparison with plasma measurements after four hour spot collection.

Conclusion:

Plain tubes are a better option for collection and processing blood in measurement of glucose if the separation is achieved within a reasonable time period.

Background

Diabetes Mellitus (DM) is a common metabolic derangement debilitating populations in both affluent and less-affluent societies alike. It is characterized by high blood glucose levels over a prolonged period[1]. Blood glucose analysis is vital for both the diagnosis and monitoring of therapy in patients with DM. The current diagnostic limits of DM are narrow and therefore there is an increased need for reliable results to classify individuals accurately[2].

In the routine laboratory set-up blood specimens for glucose determination are being collected into tubes that contain sodium fluoride (NaF). In 1941, NaF containing tubes were introduced into laboratory practice for blood collection in determination of glucose[3]. It is known that NaF shows its antiglycolytic effect by inhibiting the enolase enzyme of the glycolytic pathway in erythrocytes[4]. However, it has been recognized that the action of NaF towards enolase is slow as it will start at least in four hours from the time of collection of blood[4,5]. Therefore, Al-Kharusi et al., and Fernandez et



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al., reported that there was no difference in blood glucose values in serum separation tubes (SST) and NaF tubes which were separated within two hours of collection[3,5]. The potential disadvantage of NaF tubes has been reviewed by the American Diabetic Association (ADA) laboratory guidelines for the diagnosis and management of DM with its recommendation to stop use of NaF as the only antiglycolytic agent[2].

It has been further pointed out that the tubes containing NaF are useful if there is a delay in the separation of plasma from cellular components for several hours[5,6]. Studies done by Chan et al., however, showed that following four hours, the concentration of glucose in whole blood in the presence of fluoride remains stable up to seventy two hours at room temperature and recommended the use of NaF if there is a delay in separating[7]. Considering the slow activity of NaF towards enolase enzyme, the use of NaF tubes to collect blood for this core biochemical test has to be revisited[4,6].

In turn, the specimens collected into the tubes containing NaF are not suitable for the measurement of other key analytes such as Na⁺, K⁺ and enzymes, and therefore multiple different collection containers are required.

Objectives

The objective of this study is to compare glucose values obtained using plasma NaF/K₂C₂O₄ tubes and serum plain tubes in a routine laboratory setup, and to assess the changes in glucose levels in plasma and serum up to six hours from the separation of cellular components. Collection of blood into plain tubes is more convenient especially when it is collected from critical care patients where multiple blood samples are needed. On the contrary, the use of the historical tubes (NaF as the preservative) is not revised though there is a great advancement in the technology today.

Materials and methods

Subjects

The study was conducted at the central laboratory of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka, during a period of six months (from March to September 2017). Sixty one participants (healthy volunteers) were enrolled after written consent. The study was ethically approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (Protocol approval No. MLS/08/2017).

Methods

During the study period, blood samples (2.5 ml) were collected into both plain and NaF/K₂C₂O₄ tubes. Following centrifugation of plasma (NaF/K₂C₂O₄) and serum (plain tube), the centrifuged tube sets were allowed to stand for 1, 2, 4 and 6 hours. Glucose concentrations were measured in both plasma and serum by glucose oxidase method (DiaSysGOD FS reagent kit) using Shimadzu UV 1601 visible spectrophotometer (Simadzu Corporation, Kyoto, Japan, % CV, 0.81) [8]. For both plasma (in NaF/K₂C₂O₄ tubes) and serum (in plain tube) samples, five glucose determinations were made based on the serum separation time. At each point of analysis the serum or plasma was in contact with the blood cells in the corresponding tube. Those five determinants were baseline glucose values and glucose concentration at one, two, four and six hours after serum or plasma separation. Samples which did not show haemolysis were included in the comparison.

Each day before sample processing, internal quality control was performed using two reference glucose solutions with higher and lower glucose concentrations. The reference glucose values were 290.9 ± 10 mg/dl (high) and 94.4 ± 10 mg/dl (low) respectively. Meanwhile, the control glucose solution was also run in each day to ensure the reproducibility of the spectrophotometer. All the reagents were kept inside the refrigerator at 0°C to 8°C until the samples were processed. The reference glucose samples were aliquoted into eppendorf tubes and they were kept at -20°C.

Statistical analysis

The data are represented as mean±SD for continuous variables. A student's t-test was applied for comparison of group means. p<.05 was considered statistically significant.

Results

Glucose concentrations observed in plasma (NaF/K₂C₂O₄) and serum (plain tubes) up to six hours post separation and the percentage reduction in comparison with baseline value are depicted in Table 1. Comparing plasma (NaF/K₂C₂O₄) and serum (plain tube) glucose values (n=61, paired samples t-test) showed no significant difference up to two hours of separation (P>.05). In fact, blood specimens collected into plain tubes are much better since they had shown minimal reduction up to two hours post separation. Nevertheless, glucose concentrations measured after four and six hours in plasma and serum showed a significant difference (p<.001). Furthermore, a greater reduction was

observed with serum glucose values four and six hours post-separation in the blood samples drawn into plain tubes. Blood collected to the NaF/K₂C₂O₄ tubes showed a higher rate of hemolysis (11%) when compared with the samples collected to plain tubes (4%).

Table 1. Mean glucose concentrations of plasma (NaF/K₂C₂O₄) and serum (plain tube) and percentage reduction of glucose values with respect to baseline; post separation up to six hours

	Plasma glucose (mmol/l) mean±SD	Percentage reduction in plasma	Serum glucose (mmol/l) mean±SD	Percentage reduction in serum
Baseline	6.3±2.7*	-	6.2±2.7*	-
1 hour	6.1±2.6*	3	6.2±2.7*	0
2 hour	5.9±2.6*	6.3	6.1±2.7*	1.6
4 hour	5.7±2.7**	9.5	5.0±2.6**	19
6 hour	5.6±2.6**	11.1	4.7±2.4**	24

Analysed by paired sample t-test

*Mean glucose values in NaF/K₂C₂O₄ and plain tubes are not significantly different (p>.05)

**Mean glucose values in NaF/K₂C₂O₄ and plain tubes are significantly different (p<.001)

Discussion

The present study has confirmed that plain tubes should be used instead of NaF/K₂C₂O₄ tubes to collect blood for glucose measurements with accepted values under routine laboratory setup. In both types of tubes, clinically equivalent results were yielded given that the blood sample processing had an optimum time of less than two hours from collection to the time of analysis.

These results are comparable with the findings of previous investigators[3,5]. With the progress and improvements of the technology, it has been revealed that serum analyzed for glucose within reasonable time after the collection (less than two hours) gives same blood glucose values as in plasma in NaF tubes. The maximum separation time of serum/ plasma documented in American Diabetes Association (ADA) guidelines is thirty minutes from collection[2]. A significant reduction in blood glucose values collected to NaF/K₂C₂O₄ has been observed by several researchers and the difference reported was proportional to the time delay[5,9,10]. The use of these historical tubes appeared to be suitable for blood collection if there is a long delay in the separation of plasma from cells[10]. According to Chan et al., NaF has a minimal preservative effect within two hours of blood collection[7]. Its effectiveness was reported after four hours of blood

collection and activity persisted at least for three days at room temperature.

Use of plain tubes for glucose measurements, offer many specimen processing advantages; these tubes can be used for the majority of biochemistry

and immunology tests thus reducing the need for other consumables (additives) and the amount of blood drawn from the patients. If NaF/K₂C₂O₄ tubes are replaced with plain tubes, several laboratory tests including glucose can be performed from a single vial. This is especially important in critical and geriatric patients where venipuncture may be difficult. Additionally, the use of plain tubes offer significant financial saving. This is more important in state sector hospitals in countries such as Sri Lanka where free health care is offered.

Further, it was observed that, the blood samples collected in NaF/K₂C₂O₄ tubes had the higher rate of haemolysis (11%) when compared with that in plain tubes (4%). Most previous studies have shown similar results[3, 5]. Presence of larger crystals of NaF/K₂C₂O₄ could rupture the RBC resulting in the higher rate of haemolysis[11]. Release of glucose from RBC has a positive bias and release of catalase has a negative bias on actual glucose value, thus raising a controversy in validity of results[12].

Conclusion

The results of this study confirms that clinically acceptable blood glucose values could be obtained by using plain tubes instead of NaF/K₂C₂O₄ tubes if the analysis is performed within a reasonable time of less than two hours following blood collection. However, if analysis of blood glucose is delayed or expected to be delayed for more than two hours following blood collection, fluoride containing tubes could be acclaimed for glucose measurement.

Disclosures

The authors declare no conflict of interest. No funding was received for this work.

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