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Original Article

Biochemistry Section

A Comprehensive Stability Study of Glucose Concentrations Measured in Sodium Fluoride, Heparin and Serum Separator Tubes: A Cross-sectional Study

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ABSTRACT

Introduction: Sodium fluoride mixed vacutainers were once viewed as the gold standard tubes for glucose analysis. However, their ineffectiveness in inhibiting glycolysis, especially in the first one to four hours has been reported in several studies. The accurate blood glucose estimation is crucial for the diagnosis and management of diabetes mellitus.

Aim: To find out a cost effective and feasible alternative blood collection vacutainer for the estimation of accurate blood glucose.

Materials and Methods: A cross-sectional study was conducted at the Blood Collection Centre of HAH Centenary Hospital, New Delhi, India, from December 2019 to July 2020. A blood sample was collected from 155 subjects in each-sodium fluoride [Na_2EDTA], heparin [sodium heparin] and Serum Separator Tube (SST). To find out the glucose reduction during blood coagulation in SST tubes, the glucose levels (n=25) of sodium fluoride (plasma) at 0 hr and 30 mins after coagulation in SST tubes were compared. The remaining study subjects (n=130) were categorised into two groups (65 subjects in each group) and assessed for the reduction of glucose at 4°C and 25°C. Further, the reduction of glucose was also studied in both the groups as per glucose concentrations <100 mg/dL (n=25), 100-200 mg/dL (n=25) and >200 mg/dL (n=15) in each group.

Results: The inter assay coefficient of variation of quality control samples of glucose (n=61) was 3.0%. The highest percentage of glucose reduction was found in the green vacutainers ($p<0.05$) at 4°C and 25°C storage temperatures. There was no significant reduction of glucose level in SST vacutainers at 4°C up to 96 hrs of analysis ($p>0.05$) and the result was comparable to grey vacutainers. Further, there was no haemolysis in the SST vacutainers up to 96 hrs of analysis, however, green and grey vacutainers showed enormous haemolysis.

Conclusion: The glucose analysis in SST vacutainers can reduce the loss of sample, cost of analysis, and interferences in the analysis due to haemolysis. The study strongly suggests that all the analytical laboratories should do glucose analysis in SST vacutainers.

Keywords: Analytical laboratory, Auto-analyser, Blood collection vacutainers, Glucose analysis

INTRODUCTION

The incidence of diabetes mellitus in the population is very high globally [1,2]. Glucose estimation is the primary biochemical marker for screening the population for diagnosis of diabetes mellitus [3]. The analytical diagnostic laboratories of most of the countries are faced with a huge competition leading to financial loss or diminishing the quality of analysis [4]. There is a need for efficient use of financial resources. The laboratory must be able to provide the diagnostic services at the lowest possible cost without compromising the quality of the test. In India, there are approximately 60,000 registered diagnostic centres. Hypothetically, suppose we take an average of 100 glucose tests per day in each laboratory along with serum analysis, it will become a total 6000000 (sixty lakhs) glucose tests per day in India [5]. The sodium fluoride containing vacutainer (grey vial) is routinely used in analytical laboratories for glucose estimation. For the analysis of glucose along with serum based investigations, most of the laboratories use extra grey vacutainer (fluoride) along with Serum Separator Tubes (SST)/red vacutainers. Sodium fluoride inhibits the glycolytic enzyme enolase and retains the glucose level constant until analysis by inhibiting the ex vivo consumption of glucose [6]. The increased chance of haemolysis during transportation, less stability at room temperature, cost of extra vacutainer, and blood loss of the patients are the major disadvantages of grey vacutainers for estimation of glucose [7]. If serum and plasma both samples are requested for chemistry (liver/kidney function tests, etc) and glucose estimation, it is sometimes difficult to collect the samples in both the vacutainers (plasma/serum) from paediatric and Intensive Care Unit (ICU) critical patients. Nowadays, SST vacutainer is widely used for the parameters based on serum analysis. There is a restricted contact between serum and blood cells after centrifugation in SST vacutainer, therefore, it is worthwhile to scientifically validate the glucose estimation in this vacutainer to reduce the cost of investigation without affecting the quality [8]. It will directly benefit the patients and thousands of laboratories daily. Therefore, the present study was attempted scientifically to decipher the alternative/cost-effective blood collection vacutainer for glucose assay.

MATERIALS AND METHODS

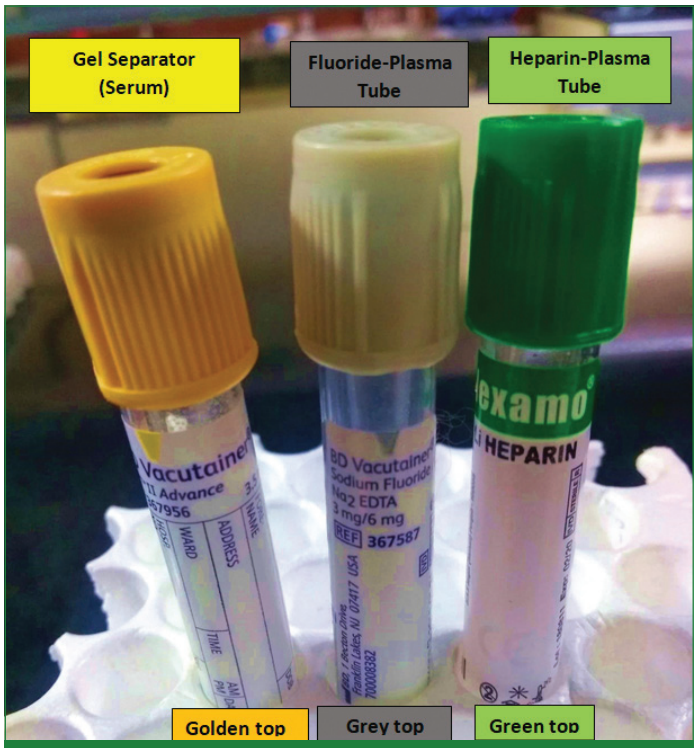
The cross-sectional study was conducted at the Blood Collection Centre of HAH Centenary Hospital, New Delhi, India, from December 2019 to July 2020 which included 155 subjects recruited randomly. Informed consent was obtained from each subject. The study was ethically approved by the Institutional Ethics Committee (IEC) of Jamia Hamdard, New Delhi, India (Ethical clearance number 12/19, meeting held on October 11, 2019).

Inclusion criteria: Patients/subjects >18 years of age, both the genders, and those who consented to participate in the study were included.

Exclusion criteria: The critically ill patients, subjects on insulin, and subjects not willing to give informed consent for participation in study were excluded from the study.

Study procedure

After overnight fasting, 9 mL of blood samples were collected from study subjects in each Becton Dickinson (BD) Vacutainer (3 mL in each vacutainer) of sodium fluoride [Na_2EDTA] (plasma); grey top, heparin [sodium heparin] (plasma); green top, and serum separator tube (serum); SST vacutainer (without anticoagulant); golden top for glucose analysis. The image of the vacutainers is given in [Table/Fig-1]. All the vacutainers were inverted for 8-10 times to mix the additive evenly with blood after sampling. The samples in upright position were transported to the laboratory in temperature controlled ice box immediately after blood collection. The blood in the SST vacutainer needed to be allowed to clot to obtain the serum sample. To find out the glucose reduction during blood coagulation, the reduction of glucose at 0 hr in sodium fluoride/ sodium heparin (plasma), and 30 mins in SST tubes was determined. Hence, samples of study subjects (n=25) in sodium fluoride tube was processed immediately after collection (0 hr glucose). Further, the sample of same study subjects (n=25) in SST tubes was allowed to undergo proper coagulation for 30 mins and then centrifuged (REMI R-8C BL at 2500 Relative Centrifugal Force for 5 mins). The remaining study subjects (n=130) were distributed in two groups (65 subjects in each group) and assessed for the reduction of glucose at 4°C and 25°C. Further, the reduction of glucose was also studied in both the groups as per glucose concentrations <100 mg/dL (n=25), 100-200 mg/dL (n=25) and > 200 mg/dL (n=15) upto 96 hrs each. The glucose level in study samples was analysed in Beckman Coulter AU480 biochemistry autoanalyser using the kits supplied by M/S Beckman Coulter. The analysis of glucose was carried out by using the 'hexokinase method' [9]. Glucose levels in grey vautainers (sodium fluoride) were used as controls for this study. Two levels of quality control (Bio-Rad) samples [level 1(low) and level 2 (high)] were analysed daily before the analysis of glucose.



[Table/Fig-1]: Blood collection vacutainers used in the study.

STATISTICAL ANALYSIS

For normally distributed each variable (n>30), values were expressed as Mean±Standard Deviation (SD). Data were compared with baseline (30 mins) to subsequent intervals of analysis (2, 4, 8, 24, 48, 72, and 96 hrs) using 'two way Analysis of Variance (ANOVA), multiple comparison (n=65) [Bonferroni post tests]. For non-normal distributions (if <30 subjects), the value was presented as mean/ median (Range), and analysis was carried out using Wilcoxon and Friedman test. Further, the difference of glucose in plasma and serum samples was evaluated by 'paired t-test'. The statistical analysis was carried out in Graph Pad Prism, ver. 5.0 software. At 95% confidence interval, p<0.05 was considered as statistically significant.

RESULTS

A total of 166 subjects were randomly selected for eligibility in this study, out of which, 11 subjects were excluded as they did not give consent to participate. The remaining 155 subjects were distributed between group 1 (n=25), group 2 (n=65), and group 3 (n=65). The demographic characteristics of the study subjects are shown in [Table/Fig-2]. The percent Coefficient of Variation (% CV) of control 1 (low) and control 2 (high) [Bio-Rad Lyphochek assayed chemistry control] was 3.4% and 2.5%, respectively. Further, the inter assay CV of quality control samples of glucose [low/high] (n=61) was 3.0%. There was no significant difference in glucose level at 0 hr of grey and 30 mins of SST vacutainers (p=0.056) and 30 mins of grey and 30 mins of SST vacutainers (p=0.065; Wilcoxon Signed Rank Test) [Table/Fig-3].

Characteristics	Test groups				
	Total subjects (n=155)	Group 1* (n=25)	Group 2# (n=65)	Group 3@ (n=65)	p-value
Average age; years (Mean±SEM) (Range)	29.8±0.98 (21-50)	31.5±1.4 (20-53)	30.7±0.10 (18-52)	30.6±1.5 (18-55)	>0.05
Male (%)	61.5	58.8	58.8	54.7	-
Female (%)	38.5	41.2	41.2	45.3	-
BMI; Kg/m^2 (Mean±SEM)	24.2±0.60	23.9±0.52	23.9±0.57	23.3±0.49	>0.05
Clinical history of study subjects					
Diabetes mellitus, n (%)	92 (59.4)	14 (56)	39 (60)	38 (58.5)	-
Miscellaneous (ortho/surgery/ eye/ ENT etc), n (%)	29 (18.7)	5 (20)	12 (18.5)	13 (20)	-
Healthy subjects, n (%)	34 (21.9)	6 (24)	14 (21.5)	14 (21.5)	-

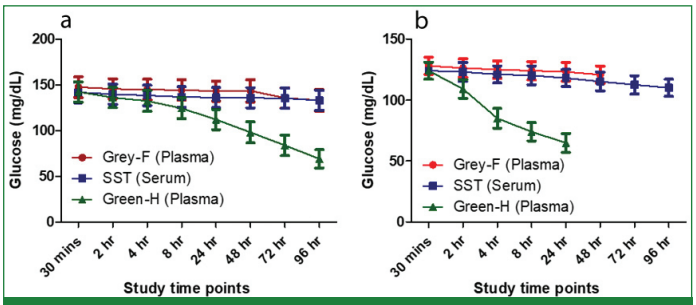
[Table/Fig-2]: Demographic characteristics of study subjects.
BMI: Body mass index; Data are expressed as number, percentage and mean±SEM; The difference of two groups was statistically assessed by using unpaired 't-test' and p<0.05 was considered as significant difference; p>0.05; No statistical difference; *group 1: Blood samples were collected to assess the reduction of glucose in SST tubes during coagulation process; #Blood samples were collected in each grey, green, and SST vacutainers and stored at 4°C to assess glucose reduction up to 96 hrs @; Blood samples were collected in each grey, green, and SST vacutainers and stored at 25°C to assess glucose reduction up to 96 hrs

	0 hr Glucose Mean (mg/dL) [median (Range)]	30 mins Glucose Mean (mg/dL) [median (Range)]	p-value (0 hr (grey) to 30 mins	p-value (compare with 30 mins (grey)	% reduction of glucose as compared to standard (grey) (0 hr- 30 mins)
Grey-F (Plasma)	114.4 [105 (84-192)]	113.5 [105 (82-190)]	-		-
SST (Serum)	-	111.6 [102 (83-190)]	0.056	0.065	2.4
Green-H (plasma)	113.4 [104 (83-191)]	110.5 [103 (80-189)]	0.010	0.035	3.4

[Table/Fig-3]: Percent reduction of glucose (0 hr- 30 mins) in SST vacutainers as compared to grey vacutainers (n=25), samples stored at 25°C (room temperature).
Grey-F: Fluoride containing vacutainer; SST: Serum separator tube (vacutainer); Green-H: Heparin containing vacutainer; Glucose levels in SST and green vacutainers at 30 mins of analysis were compared with glucose level in grey vacutainers (standard) at 0 hr; Wilcoxon signed rank test was used to calculate the p-value; p>0.05 (not significant); p<0.05 (statistically significant)

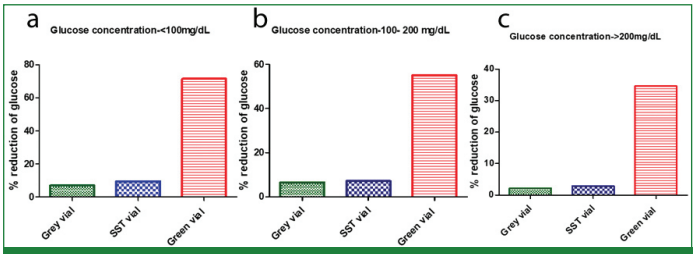
The reduction of glucose in grey, green, and SST vacutainers, samples stored at 4°C (n=65) and 25°C (n=65) is shown in [Table/Fig-4]. The reduction of glucose in SST vacutainers at 4°C were not statistically significant up to 96 hrs of analysis (p>0.05, n=65) and the results are comparable to grey vacutainers. However, the

glucose reduction in green vacutainers (heparin) showed a significant reduction at the 72 hrs of analysis (p<0.001). Furthermore, at 25°C (room temperature), the glucose level was not detected in grey and green vacutainers at 48 hrs and 24 hrs of analysis respectively, due to gross haemolysis in the samples. Interestingly, there was no haemolysis in SST vacutainers till 96 hrs of analysis. Moreover, the reduction of glucose in SST and grey vacutainers at 48 hrs was not statistically significant (p>0.05, n=65). Though, in green vacutainers, the reduction of glucose was statistically significant at 4 hr of analysis (p<0.001).



[Table/Fig-4]: (a) Mean reduction of glucose (30 mins- 96 hrs) at 4°C (n=65) in grey (plasma), SST (serum), and green (plasma) vacutainers. Grey-F; Fluoride containing vacutainer, SST; Serum separator tube (vacutainer), Green-H;Heparin containing vacutainer. Data were compared with baseline glucose level (30 mins) of respective group and grey vacutainer at different time points (2, 4, 8, 24, 48, 72, and 96 hrs) using 'two way' ANOVA, multiple comparison (n=65) [Bonferroni post-tests] p-value: Grey vs SST p> 0.05 (ns), Grey vs green- H (2, 4, 8, 24) p>0.05 (ns), Grey vs green- H (48 , 72, and 96 hrs) p<0.05 (significant). (b) Glucose reduction (30 mins- 48 hrs) at 25°C (n=65) in grey (plasma), SST (serum), and green (plasma) vacutainers. Grey-F; Fluoride containing vacutainer, SST; Serum separator tube (vacutainer), Green-H; Heparin containing vacutainer Statistics: Grey vs SST (2,4,8,24, and 48 hrs) p> 0.05 (ns), Grey vs green- H (2,4,8,24 hrs) p<0.05 (significant). Glucose level was not detected in grey and green vacutainers at 48 hr and 24 hrs of sample collection, respectively due to the enormous haemolysis

The authors have further categorised the study subjects of group 2 (n=65) and group 3 (n=65) in three sub-groups based on glucose concentration (<100, 100-200, >200 mg/dL) [Table/Fig-5] (group 2) and [Table/Fig-6] (group 3). The reduction of glucose in grey and SST vacutainers at different concentration of glucose (at 4°C) in different time intervals was studied using repeated measures one-way ANOVA (Friedman test) and the reduction was not statistically significant [p>0.05 (ns)]. However, the reduction of glucose in grey and SST vacutainers is statistically significant at 25°C [p<0.05 (sig)]. The reduction of glucose in SST vacutainers is similar to the grey vacutainer. Thus, the decline of glucose in SST vacutainers at 4°C and 25°C in different time intervals is comparable to the grey vacutainers.



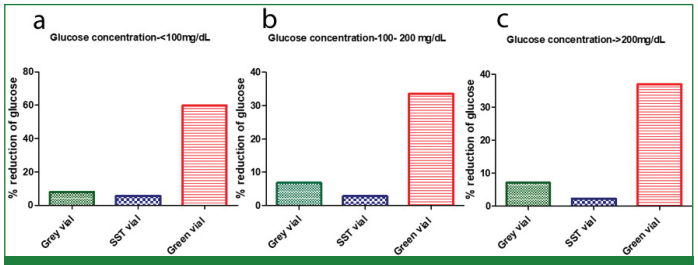
[Table/Fig-5]: Percent mean reduction of glucose in group 2 in Grey-F; Fluoride containing vacutainer, SST; Serum separator tube (vacutainer), Green-H; Heparin containing vacutainer, stored at 4°C for different glucose concentrations (a) <100mg/dL (n=25); the reduction of glucose in vacutainers in different time intervals was studied using repeated measures one-way ANOVA [Friedman statistic: 13.79, p=0.0550 (ns)]; (b) 100-200 mg/dL(n=25); Friedman statistic: 13.29, p=0.0654 (ns), and (c) >200mg/dL (n=15); Friedman statistic: 11.33, p=0.1247 (ns).

DISCUSSION

There is a need to establish an alternative blood collection vial for glucose estimation in diagnostic laboratory as scientific studies have proven that the gold-standard tube (grey top) is ineffective in its role to promptly block glycolysis [10-12]. Moreover, uses of sodium fluoride containing vacutainer for glucose analysis is not recommended by the American Diabetes Association [3]. The present study validates that no significant reduction of glucose in SST tubes and levels of glucose are comparable to sodium fluoride vacutainer (grey). Interestingly, the study outcome is similar to the outcome of the studies of Fernandez L et al., 2013 [13], Al-Kharusi A et al., 2014 [7] and Dibbasey M et al., 2022 [14] in which they qualify both tubes to be suitable candidates for glucose analysis.

Earlier studies have shown that the gel SST has a potential to be used as an alternative vacutainer for glucose analysis [6,8]. The present study differed from other past studies because each tube was comprehensively allowed to stand for various storage times (2,4,8,24,48,72, and 96 hrs) and at different temperatures (4°C and 25°C (room temperature)), then assayed the respective samples for their glucose level. An earlier study had reported that glucose levels in grey vacutainers that contain an inhibitor of glycolysis, were significantly higher as compared to heparin-containing tubes [15]. These results extend to 4 hours, at which time the fluoride is thought to have exerted its maximal inhibitory effect on the glucose metabolism in red blood cells [11]. In the present study, the plasma glucose level in green vacutainers (heparin) was significantly decreased at 2 hrs of analysis. Li G and Cabanero M, 2013 [16] reported that glucose level was decreased in serum samples (red top vacutainer) at 4 hrs of analysis. Though, in the present study, there was no significant reduction of serum glucose up to the 96 hrs of analysis at 4°C storage temperature, even though SST vacutainers were used for the extraction of serum. In SST vacutainer, there is restricted contact between cells and serum. Our results showed that the reduction of glucose levels in grey and SST vacutainers were very similar, whereas for the same time the green vacutainers (heparin) had a statistically significant reduction of glucose in the study samples. Moreover, there was no haemolysis in SST vacutainers up to 96 hrs of analysis.

Since both vacutainers (grey and SST) are suitable candidates as shown by the data [Table/Fig-3-6], preference may be given to SSTs as these can be used for a wide spectrum of biochemical analyses and increase feasibility in analysis and reduce mistakes associated with analysing both plasma and serum at the Biochemistry laboratory for an individual patient. Besides the low cost of vacutainers, elimination of grey tubes would be a significant cost-saving measure for the diagnostic laboratory as large number of laboratory-based glucose tests are performed daily. The use of only SSTs will also reduce the volume of blood collected from patients when glucose and other analytes are requested together and improve the laboratory workflow.



[Table/Fig-6]: Percent reduction of glucose in group 3 in Grey-F; Fluoride containing vacutainer, SST; Serum separator tube (vacutainer), Green-H; Heparin containing vacutainer, stored at 25°C for different glucose concentrations (a) <100 mg/dL; the reduction of glucose in vacutainers in different time intervals was studied using repeated measures one-way ANOVA [Friedman statistic: 7.6, p=0.0417 (sig)]; (b) 100-200mg/dL; Friedman statistic: 8.0, p=0.0083 (sig) (c) >200mg/dL; Friedman statistic: 10.0, p=0.0014 (sig).

Limitation(s)

The reduction of glucose in the present study was carried out in BD vacutainers only (single lot number). The assessment of the glucose reduction in the vacutainers of other manufacturers was a limitation of the present study.

CONCLUSION(S)

In all the vacutainers, with the possible exception of the gel barrier vacutainers (SST etc), there is some contact between red cells and plasma/serum. Despite centrifugation, there is a continuous decrease of glucose levels in the patient samples and this can

affect the obtained values if repeat analysis is requested. In the present study, the authors have analysed blood glucose reduction at different temperatures and up to 96 hrs of analysis. There was no significant reduction of glucose levels in SST vacutainers (up to 96 hrs of analysis at 4°C storage temperature) and the result was comparable to grey vacutainers. In view of study outcomes and cost reduction/feasibility of analysis without compromising on quality in glucose estimation, the authors strongly suggest that all the analytical laboratories may use SST vacutainers for glucose analysis.

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- Plagiarism X-checker: Sep 16, 2021
- Manual Googling: Jun 18, 2022
- iThenticate Software: Jun 30, 2022 (9%)

ETYMOLOGY: Author Origin

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