



COMPARISON OF FLUORIDE-OXALATE AND PLAIN TUBES ON GLUCOSE STABILITY IN BLOOD SPECIMENS

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KEYWORDS :

INTRODUCTION

Diabetes Mellitus (DM) is a major global public health burden, with an increasing prevalence worldwide. The global prevalence is expected to rise substantially in the coming decades, reaching 366 million in 2030.[1] Hyperglycaemia is the biochemical hallmark of DM and the diagnosis of which relies primarily on estimation of blood glucose levels. Plasma glucose estimation remains the cornerstone for the diagnosis of DM as well as Gestational Diabetes Mellitus (GDM), where glycated haemoglobin (HbA1c) is not recommended as a primary diagnostic test. [1,2,3]

Diagnostic thresholds for fasting plasma glucose, post-prandial and random plasma glucose are based on relatively small differences in the glucose concentrations. Therefore, even minor pre-analytical errors may result in the misclassification of patients and in turn inappropriate clinical decisions. Enzymatic assays are currently the principal analytical methods of glucose estimation and demonstrate excellent analytical precision with inter-laboratory Co-efficient of variation (CV)<2.6%. [1,4] As analytical variation is substantially minimised through standardisation, pre - analytical factors remains a major source of error, leading to the total variation in glucose estimation. Ex-vivo glycolysis during the pre-analytical period, results in loss of glucose in blood samples (5-7% per hour). [5,6]

With recognition of the impact of glycolysis on glucose concentration, the World Health Organisation recommends that glucose concentrations should not be measured on serum unless red cells are immediately removed. Similarly, the American Diabetes Association also recommends that glucose levels should be measured in plasma samples separated from cells within 60 minutes of collection. [7,8] However, in routine clinical practice, immediate sample processing is often not possible, particularly in high volume and resource limited laboratory settings. So, the problem of ex-vivo glycolysis during the pre-analytical period, resulting in progressive loss of glucose in blood samples continues. In cases where immediate sample processing is not feasible, several approaches have been adopted- centrifugation immediately after sample collection, refrigeration, use of anti-glycolytic agents such as iodoacetate, mannose and fluoride in the form of sodium fluoride – potassium oxalate tubes (NaF-KOx). [6,9-13]

NaF/KOx tubes have been traditionally regarded as the gold standard for sample collection for measurement of plasma glucose levels. However, fluoride alone is not a fast-acting inhibitor and its effect is delayed for up to 4 hours after sample collection. [14-16] NaF/citrate/EDTA tubes have demonstrated superior stabilisation of glucose concentrations. [17,18] However, their routine use is limited by availability and cost.

Several studies have reported significantly higher mean glucose concentration in Serum Separator Tubes (SSTs) compared to NaF/KOx tubes when samples are processed within recommended time limits. [19,20] Consequently, SSTs are increasingly being utilised for glucose estimation in routine laboratory practice as substitutes for

NaF/KOx tubes. However, SSTs may not be readily available in all healthcare settings, particularly in resource -limited laboratories. Plain serum tubes are widely available and inexpensive and routinely used for a number of biochemical investigations; nevertheless, the suitability of plain tubes for glucose estimation and effect on glucose stability has not been reported.

Therefore, the present study was undertaken to compare the glucose concentrations obtained from plain tubes and NaF/KOx tubes and to evaluate the effect of delayed sample processing on glucose stability. The findings may provide potential evidence for the use of a practical and cost-effective alternative to conventional glucose collection tubes in routine laboratory practice.

MATERIALS AND METHODS

Study Setting and Participants

The study was conducted in the Clinical Biochemistry Laboratory of Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal. Venous blood samples were collected from students enrolled in the MBBS and BDS courses at the institute. A total of 80 participants aged 19-23 years were recruited irrespective of the timing of their last meal, as the objective of the study was to evaluate the effect of sample tube type, separation condition, and time on glucose stability rather than to assess fasting glycaemic status.

Sample Collection and Processing

Approximately 8 mL of venous blood was collected from each participant under aseptic conditions and distributed equally into four tubes (2 mL per tube): two plain tubes and two sodium fluoride/potassium oxalate (NaF/KOx) tubes. The samples were assigned to two processing conditions: immediate separation and delayed separation.

All four tubes were centrifuged at 3000 rpm for 5 minutes within 15 minutes of blood collection. For the immediate-separation condition, serum from the plain tube and plasma from the NaF/KOx tube were physically separated from the cellular components immediately after centrifugation. For the delayed-separation condition, the corresponding plain and NaF/KOx tubes were centrifuged under the same conditions, but the serum or plasma was left in the original tube in contact with the underlying cellular components and was separated only immediately before glucose analysis at the designated time point.

Glucose concentrations were assessed at 0 hour (within 30 minutes of sample collection), 1 hour, 4 hours, and 24 hours after collection. The samples were maintained at [insert actual storage temperature/condition] between the designated analytical time points. Thus, glucose stability was evaluated according to tube type (plain versus NaF/KOx), post-centrifugation separation condition (immediate versus delayed separation), and time after blood collection (0, 1, 4, and 24 hours).

Glucose Estimation

Blood glucose concentration was measured by the glucose oxidase-

peroxidase (GOD-POD) method using the DiaSYS 200 Pro chemistry analyser. The same lot of glucose reagent was used throughout the study to minimize analytical variation.

Quality Control

Before analysis of the study samples, two levels of internal quality control material (normal and high) were analysed to verify the analytical performance of the chemistry analyser. Patient samples were analysed only after the quality control results were found to be within the laboratory's acceptable limits.

Participant Inclusion in Final Analysis

Although samples were initially collected from 80 participants, complete measurements at the prespecified time points were available for 55 participants and were included in the final analysis. Samples from the remaining 15 participants were excluded because measurements could not be completed at the required time points due to analyser availability. This exclusion was documented as a study limitation.

Data Analysis

Statistical analysis was performed using IBM SPSS Statistics version 16.0. Continuous variables were summarized as mean $\pm$ standard deviation (SD), where appropriate. Comparisons between paired measurements under specified conditions were performed using the paired-samples t-test when the assumptions for parametric testing were satisfied. Repeated-measures ANOVA was used to assess changes in glucose concentrations across the different time points. A two-tailed p-value <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted after approval from the Institutional Ethics Committee of JNIMS. Written informed consent was obtained from all participants before blood collection. Participation was voluntary, and confidentiality of participant information was maintained.

RESULTS

The results of the study are presented in the following tables and figures. The findings were analyzed to assess the changes in Mean Glucose Concentration (MGC) values with time in plain and fluoride-containing tubes.

Table 1: MGC (mg/dl) with Time, in Immediately Separated Sample Put on Different Tubes.

Time (hours)	Plain tube (immediately separated)	NaF/KOx tube (immediately separated)	t-test	p-value
0	115.2 $\pm$ 48.7	113.2 $\pm$ 49.6	1.16	0.254
1	113.2 $\pm$ 47.5	112.0 $\pm$ 50.4	0.614	0.0544
4	112.5 $\pm$ 47.9	112.1 $\pm$ 48.8	0.231	0.819
24	108.9 $\pm$ 51.0	111.2 $\pm$ 48.6	1.485	0.147

Table 1 shows that Mean glucose concentration (MGC) shows no significant difference between plain and fluoride tubes at all time points when samples were immediately separated. No significant differences were observed up to 24 hours, indicating effective prevention of glycolysis by prompt plasma separation. The consistency across all intervals confirms that both tubes types provide reliable glucose estimates under immediate processing conditions.

Table 2: MGC (mg/dl) with Time, Put on Different Tubes and Different Time of Sample Processing.

Time (hours)	Plain tube (immediately separated)	NaF/KOx tube (not immediately separated)	t-test	p-value
0	115.2 $\pm$ 48.7	112.5 $\pm$ 50.0	2.063	0.047
1	113.2 $\pm$ 47.5	111.8 $\pm$ 48.9	0.804	0.427
4	112.5 $\pm$ 47.9	110.8 $\pm$ 47.5	0.775	0.444
24	108.9 $\pm$ 51.0	99.9 $\pm$ 50.0	3.637	0.001

The present table also demonstrates that glucose concentrations begin to diverge when fluoride tubes are not immediately separated. Although early differences were minimal, a statistically significant drop appeared at 24 hours ($p<0.05$) in delayed-separation fluoride samples. This indicates that delayed processing allows glycolysis to continue before fluoride inhibition becomes effective, leading to lower glucose values over time.

Table 3: MGC (mg/dl) with Time, Put on Different Vial and not Immediately Processed.

Time (hours)	Plain tube (not immediately separated)	NaF/KOx tube (not immediately separated)	t-test	p-value
0	115.2 $\pm$ 48.7	112.5 $\pm$ 50.0	3.352	0.002
1	111.6 $\pm$ 47.5	111.8 $\pm$ 48.9	0.163	0.872
4	105.5 $\pm$ 48.8	110.8 $\pm$ 47.5	3.646	0.001
24	71.1 $\pm$ 55.2	99.9 $\pm$ 50.0	9.207	0.000

Table 3 reveals pronounced effects of delayed processing on glucose stability, especially in plain tubes. Glucose levels declined significantly from 4 hours ($p<0.05$) onward and dropped drastically at 24 hours ($p<0.05$) in unseparated plain samples. Fluoride tubes showed comparatively better stability in case of delayed sample processing.

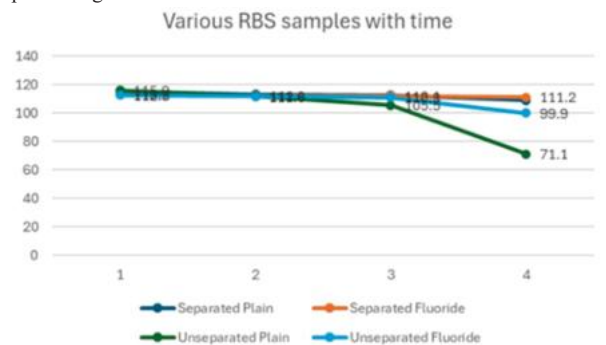


Fig 1: Graph Showing Variation in the Stability of Glucose Measured in NaF/KOx and Plain Tubes with Time.

The above figure illustrates clear differences in glucose stability based on tube type and processing time. Both immediately separated groups displayed stable glucose levels throughout. Unseparated fluoride samples showed moderate decline, while unseparated plain samples demonstrated severe glucose loss, especially at 24 hours. The trends visually reinforce that immediate separation is critical for maintaining accurate glucose concentrations.

DISCUSSION

The present study demonstrated no significant differences in the glucose concentrations between NaF/KOx tube and plain tube when the samples were processed immediately after collection. However, significant differences emerged with delayed processing, indicating that the effect of the collection tube on the glucose stability depends primarily on the interval between sample collection and sample processing.

The absence of a significant difference in glucose concentration between immediately processed plain tube and NaF/Kox tubes observed in the present study is consistent with the outcome of the studies conducted by Fernandez et al. [20] and Al-Kharusi et al. [21] who also reported comparable glucose values when samples were centrifuged and separated promptly after collection. These observations indicate that prompt separation of serum from blood is the most important determinant of glucose stability, irrespective of the collection tube used and further support the presence of antiglycolytic additive. The agreement between the present study and previous reports may be attributed to the effective prevention of ex-vivo glycolysis following early separation of serum from blood. Still, both the collection tubes provide comparable glucose results if centrifugation and separation of plasma and serum are done within 30 mins of sample collection as recommended by the ADA while some studies suggested separation to be done within 1 hour.[8]

Although Na/F inhibits enolase in the glycolytic pathway, it's antiglycolytic action is not immediate, allowing continued glycolysis during the initial post-collection period, as evident from the findings of this study. It was observed that immediately centrifuged plain tube had a higher glucose concentration than NaF/KOx tube which was not immediately separated; the difference was highly significant at 24 hours ($p=0.001$). These findings were in agreement with observations of Gambino [18] and Waring et al. [22] This phenomenon can be explained by the inhibition of enolase which occurs relatively late in the glycolytic pathway. These findings corroborate previous reports demonstrating that NaF/KOx tubes alone cannot completely prevent early glucose loss.

The present study demonstrated significantly higher glucose concentration in NaF/KOx tubes as compared to plain tubes at 4 hours and 24 hours when samples were left unprocessed until analysis. Similar observations have been reported by Amegashie et al. [23], documenting the superior glucose stability in NaF/KOx tubes during prolonged storage. The higher glucose concentration may be attributed to inhibition of glycolysis over time. These findings highlight the clinical utility of NaF/KOx tubes in settings where immediate centrifugation is not feasible. Therefore, while plain tubes are suitable for glucose estimation when rapid processing is guaranteed, NaF/KOx tubes remain the preferred collection tube when delays in transportation or processing are anticipated. As such, the findings of the present study indicate that plain tubes can serve as a reliable alternative when prompt serum separation and processing are ensured.

The findings of the present study have important implications for routine clinical laboratory practice. The results suggest that plain serum tubes provide glucose measurements comparable to those obtained with NaF/KOx tubes, provided that serum or plasma is separated promptly after blood collection. Since plain serum tubes are routinely used for multiple blood investigations, the same specimen may also be used for glucose estimation, thereby eliminating the need for additional NaF/KOx tubes in appropriately processed samples. This approach may reduce consumable cost, simplify laboratory workflow and reduce biomedical waste. Nevertheless, when prompt sample processing cannot be ensured as commonly encountered in high volume laboratories, peripheral collection centers or resource constrained healthcare facilities - NaF/KOx tubes remain the preferred collection tubes because of superior ability to preserve glucose concentration during prolonged storage, thereby minimizing the pre-analytical glucose loss.

Certain limitations of our study should be acknowledged. The effect of haemolysis on the glucose stability was not evaluated. As reported by Al-Kharusi et al. [21] and Ko et al. [24], high hemolysis rates in NaF tubes than in plain tubes could potentially influence glucose concentration due to effect of catalase, resulting in the slight decrease in NaF mean glucose concentration by 0.06 mmol/L. [25] In addition, this was a single center study with limited sample size which may affect the generalizability of the findings.

Future multicentric studies involving larger populations, assessments of additional anti-glycolytics such as citrate and the effects of potential confounders such as diabetic status, underfilling or overfilling are warranted to validate the findings and evaluate applicability in routine laboratory practice.

CONCLUSION

In conclusion, the findings of this study indicate that, the key determinant of glucose stability is prompt sample processing rather than the type of tube used. Plain tubes performed comparably to NaF/KOx tubes with prompt separation, whereas NaF/KOx tubes provide better glucose preservation when processing is delayed. Appropriate tube selection and adherence to recommended pre-analytical practices are essential for reliable glucose estimation.

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