# Validation of Fluoride Oxalate against Iodide Oxalate, Chloride Oxalate, and Glucomedics Anticoagulants for Glucose Estimation

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## Abstract

**Objective**: Accurate measurement of glucose is critical for diabetic care. Sodium fluoride/potassium oxalate (NaF/KOx) has been the preferred tube for measuring glucose. The pre-analytical challenges associated with the use of NaF/KOx and the emergence of COVID-19 presented challenges in the purchase of preservatives for measuring glucose. The need to validate other available and accessible local preservatives for measuring glucose becomes necessary. This study aimed to validate glucose values obtained using standard NaF/KOx anticoagulant against iodide oxalate, chloride oxalate, and glucomedics anticoagulant.

**Methods**: Blood samples were collected from 45 apparently healthy individuals and distributed into four tubes: NaF/KOx, sodium chloride/potassium oxalate (NaCl/KOx), iodide oxalate/potassium oxalate (IOx/KOx), and glucomedics. Samples were separated into aliquots and stored for various durations before centrifugation. Glucose analysis was measured using the glucose oxidase-peroxidase method. Statistical analysis included bias comparison, mean concentration comparison, Deming's regression, and Bland-Altman analysis.

**Result**: There was a significant decrease in glucose concentration with increasing separation time. Glucomedics showed minimal decrease, exhibited the least bias in all the time points considered with only 1-hour delayed measurement having a clinically acceptable bias of 1.62<2.2%; and demonstrated the strongest correlation with other methods. Mean concentration differences were comparable between glucomedics and NaF/KOx.

**Conclusion**: The three different anticoagulants could be a good replacement for NaF/Kox. However, glucose values obtained using glucomedics could give a better clinically useful result than others when a delay in sample processing is inevitable. The need to consider the use of any of the anticoagulants in place of NaF/KOx is strongly recommended.

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**Running Title:** Effects of Different Preservatives on Glucose Stability and Estimation.

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## Introduction

Plasma glucose estimation is one of the biochemical tests that are used to diagnose diabetes mellitus (DM), impaired glucose tolerance (IGT), and in particular for the screening and diagnosis of gestational diabetes mellitus (GDM) where glycated heamoglobin (HbA1c) measurement is not available or affordable [1]. It is also one of the most commonly measured components of blood, because of the central role of glucose in metabolism and the high prevalence of the disease of glucose homeostasis [2]. The impact of this non-communicable disease in terms of its diagnosis and management by different levels of health care providers has made measurement of glucose among the most commonly requested core biochemistry tests worldwide both as laboratory-based or point-of-care testing [3]

Blood coagulates by the transformation of soluble fibrinogen into insoluble fibrin. To achieve this, anticoagulants are used. Anticoagulants are compounds used to prevent the clotting of blood. Glucose estimation using plasma or whole blood requires the use of an anticoagulant. When blood is collected, the cells do not die immediately, as cellular metabolism is an ongoing process thereby using glucose as a source of energy via the glycolytic process. This causes glucose to disappear from whole blood on standing over a period of time [4]. However, glycolysis can be prevented with an enzyme inhibitor. The commonest inhibitor for this purpose is sodium fluoride which is usually used in conjunction with an anticoagulant potassium oxalate [5]

The use of sodium fluoride/potassium oxalate (NaF/KOx) containing tubes for the collection of samples meant for glucose estimation was introduced in the clinical chemistry laboratory in 1941 [6]. Sodium fluoride has been known to have anti-glycolytic effect. It inhibits the process of glycolysis which occurs in red blood cells. The use of this tube appears to be suitable for blood collection but has been reported to be associated with some challenges especially when there is a long delay in separating various components of blood following collection [3][4][7]. Glucose is unstable in whole blood and inaccurate glucose estimations lead to errors in the diagnosis, classification, treatment, and assessment of the risk of developing diabetes [7].

Fluoride oxalate inhibits enolase, which is far downstream in the glycolytic pathway while enzymes upstream of enolase

remain active and continue to metabolize glucose until substrates are exhausted. Thus, the anti-glycolytic action of fluoride is delayed for up to 4 hours and has little or no effect on the rate of glycolysis during the first 1-2 hours after blood is collected thereby resulting in a drop in glucose levels as much as 10 mg/dL (0.6mmol/l) precipitated by delay following sample collection [8][9].

The traditional use of NaF/KOx alone is unsatisfactory as it does not begin to inhibit glycolysis until 90-120 min has elapsed after blood is collected thereby resulting in falsely low plasma glucose concentrations and failure to detect accurate diabetics patients [10]

While laboratories make efforts to improve the analytical procedures for plasma glucose, loss of glucose from blood samples before analysis remains a threat to the accuracy of results. Eliminating this problem requires the use of an anti-glycolytic agent that can be added to the sampling tubes without altering cellular integrity while measuring blood glucose. It is obvious that when excessive loss of glucose is prevented, it will result in patients being more accurately diagnosed and managed promptly which can lead to benefits, reduce risks of diabetic complications, and equally lead to confidence in the results over prolonged periods of storage before analysis is completed.

Previous studies conducted by [11][12][13], have reported that sodium fluoride oxalate tubes were not a good inhibitor of glycolysis. Other preservatives used to prevent glycolysis such as iodoacetate which inhibits glyceraldehyde 3 phosphate dehydrogenase, also take up to 3 hours to become effective [14][15] Current blood collection tubes (lithium heparin, plain bottles, sodium fluoride/potassium oxalate (NAF/KOx)) do not immediately prevent clinically significant glycolysis, thus glucose consumption continues by different blood cells (red, white and platelets), [2][16][17]

During the COVID-19 pandemic, clinical laboratories across countries experienced high pressure precipitated by patients needing medical attention. High demand for clinical laboratory items caused serious distortion in the supply and logistics of laboratory consumables [18][19]. Supplies of laboratory consumables including sample separation tubes were limited and this affected laboratories in resource-limited settings. Furthermore, pre-analytical issues associated with the use of NaF/KOx for measuring glucose require that potential preservatives that could give more reliable, accurate, and reproducible glucose values be evaluated. The need to repurpose and validate some laboratory chemicals that are easily available and accessible for use as anticoagulants for measuring glucose, especially within an environment where budgetary allocation for the purchase of laboratory consumables is limited. This study aimed to validate glucose values obtained using standard sodium fluoride oxalate anticoagulant against iodide oxalate, chloride oxalate, and glucomedics anticoagulant.

Although researchers have not used chloride oxalate and glucomedics as an anti-glycolytic agent in this locality we assume that it may behave differently from the previously mentioned anticoagulants. An effective inhibitor of glycolysis is required to enable laboratories to estimate true glucose levels in blood. This issue needs to be addressed in an integrated manner so that the pre-analytical conditions for glucose estimation can be standardized in a suitable, efficient, and cost-effective manner. Estimation of true plasma glucose level is important not only for the diagnosis of diabetes at the earliest but also for identifying correctly high-risk patients [20].

## Materials and Methods

### Study area

This study site was University College Hospital (UCH), Ibadan

### Study design

It is a laboratory-based quantitative cross-sectional study.

### Study population

Comprised of 45 apparently healthy individuals within the age range 25-50 years who are staff of University College Hospital, Ibadan.

### Sample Size Determination

The number of participants used for the study was calculated using the following formula[21]:

$$n = \frac{(Z_\alpha)^2 SD + SD}{d^2}$$

Where:

- $n$ = number = number of participants needed
- $Z_\alpha$ = standard normal deviate corresponding to 2 sided level of significance at 5% = 1.96
- $SD$ = standard deviation 0.15, [22]
- $d$ = level of precision, d (how close to the proportion of interest the estimate is desired to be (e.g.within 5%)

According to [22]) in a study conducted on stability of serum/plasma glucose for the diagnosis of diabetes mellitus at the Ebonyi State University Teaching Hospital (EBSUTH), Abakaliki, Nigeria reported a standard deviation 0.15. Therefore, the number of subjects to be used is calculated thus

$$n = \frac{(Z_\alpha)^2 SD * SD}{d^2}$$

$$= \frac{(1.96)^2 .15 * .15}{(0.05)^2} = 34.5744$$

Adjusting for non-response rate (10%) was calculated using the following formula $= n * \frac{1}{1-r}$

Where:

- $n$ = sample size calculated
- $R$ = response rate (10%)

  = $34.57^{*} 1/(1 – 0.1)$

  = 38.4

Therefore, the minimum number of participants needed for each group is 38. However, This was adjusted to 45 participants for each group.

## Sample Collection

A total of 12 mls of random blood samples was drawn from each of the participants and 3 mls of blood was dispensed into the four different anticoagulants tubes:- sodium fluoride/potassium oxalate (NaF/KOx), sodium chloride/potassium oxalate (Nacl/KOx), Iodide oxalate/potassium oxalate (IOx/KOx) and glucomedics. The whole blood samples collected into the various tubes were immediately dispensed into 5 aliquots each containing 0.6 mls of whole blood and were labeled 0h,1h,2h,4h,24h, for NaF/Kox, Nacl/Kox, IOx/KOx and gulcomedics respectively. All labeled sample aliquots were kept at room temperature (20-25°C). After a waiting period of 0, 1,2, 4 and 24hour, they were centrifuged at 3000 rpm for 10minutes to obtain complete separation of plasma. The harvested plasma was stored in the freezer until analysis. Glucose levels in the samples (plasma) were measured manually in duplicates using the glucose oxidase-peroxidase method along with controls.

## Exclusion criteria

Patients with diabetic were excluded from the study

## Sampling technique

Simple random sampling

## Analytical procedure

Principle of glucose oxidase-peroxidase method

Glucose oxidase (GOD) catalyzes the oxidation of glucose to give hydrogen peroxide ($H_2O_2$) and gluconic acid. In the presence of the enzyme peroxidase (POD), the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminophenazone(4-aminoantipyrine) and phenol to give a pink color. The absorbance of the color produced is measured using a spectrophotometer at 500nm [23] and is directly proportional to the amount of glucose present in the sample.

**Table 1.** Preparation of Anticoagulants

| Nos | Name of Anticoagulant | Constituents | Preparation |
|---|---|---|---|
| 1. | Sodium flouride/Potassium Oxalate | Sodium fluoride 1.2 g and potassium oxalate 6.0 g | The constituents were dissolved in 100 mL distilled water. Prepared anticoagulant (0.05 mL) per ml of blood was used |
| 2. | Sodium Chloride/Potassium Oxalate | Sodium chloride 1.2 g and potassium oxalate 6.0 g | The constituents were dissolved in 100 mL distilled water. Prepared anticoagulant (0.05mL) per ml of blood was used |
| 3. | Sodium iodide/Potassium Oxalate | Sodium iodide 1.2 g and potassium oxalate 6.0 g | The constituents were dissolved in 100 mL distilled water. Prepared anticoagulant (0.05 mL) per ml of blood was used |
| 4. | Glucomedics | Citric acid 3.4 g trisodium citrate 1.6 g disodium ethylene diamine tetraacetic acid 4.8g and sodium flouride 0.2 g | The constituents were dissolved in 100 mL distilled water. Prepared anticoagulant (0.05 mL) per ml of blood was used |

### Data Analysis

Statistical Package for Social Sciences (SPSS) version 16.0 statistical software was used for analysis of data. Mean and standard deviation was calculated and Shapiro-Wilk normality test was conducted to assess if the normality distribution of the data sets was satisfied. Values of glucose concentration obtained were presented in the median and interquartile range. The trend of the concentration measured over time points by the different tubes was shown with a line graph. The difference in baseline measurement and the respective measurement taken at different time points (1,2,4 and 24 hours) for each tube was investigated using the Wilcoxon sign rank test. The clinical significance difference from the baseline concentration was examined using the expression:

Bias={(CONCt-CONCb)/CONCb}*100, for t=1,2,4 and 24hours

Where CONCb represents value of baseline concentrations measured (obtained as the mean of all measurements) and CONCt represents the value of delayed concentration measured at time t.

Difference in mean concentration amongst the tubes considered was investigated using the Kruskal-Wallis test and pairwise post-hocs test if significant. The method comparison was done using Deming regression and Bland-Atman analysis was used to assess the comparability between sodium fluoride/potassium oxalate and other anticoagulants. All analysis was conducted at 5% level of significance.

### Ethical Approval

Ethical approval for the study was obtained from Oyo State Ethical Review Committee, with approval numbe**AD 13/479/2043B**. All ethical principles were maintained during the study:

## Results

Data showed a positive relationship between fluoride oxalate and iodide oxalate anticoagulants and for every increase in

Y, there is a corresponding increase in X by 0.93. The 95% confidence interval (C.I) for X ranges between 0.88 to 0.98 of which 1 is not inclusive, this implies their level of relationship is not perfect (figure 1).

A comparative relationship between fluoride oxalate and chloride oxalate anticoagulants showed a positive relationship. For every increase in Y, there is a corresponding increase in X by 0.80. The 95% CI for X ranges between 0.73 to 0.86 of which 1 is not inclusive, this implies their level of relationship is not perfect (figure 2). There is also a strong relationship between fluoride oxalate and glucomedics anticoagulants; and for every decrease in Y, there is a corresponding increase in X by 0.98. The 95% CI for X ranges between 0.90 to 1.07 of which 1 is inclusive, this implies their level of relationship is perfect (figure 3)

Data obtained from the pattern of change in median glucose concentrations measured in the four tubes at different time points showed that there was a significant decrease of glucose concentration measured at the different time points from the baseline measurement($P<0.001$) (Table 2). Bias relative to baseline measurement increased as the delayed in time-point of measurement also increased (Table 2). The recommended set bias for this study was 2.2. Bias values exceeding the desirable goal are bolded. However, it is evident that glucomedics has the least bias in all the time points considered with only 1-hour delayed measurement having a clinically acceptable bias of $1.62<2.2\%$.

Figures 4, 5 and 6 showed the data obtained from analyzing the mean differences of iodide oxalate, chloride oxalate and glucomedics anticoagulants against fluoride oxalate using Bland-Altman graph. The closer the bias to the mean, the better the agreement between the two anticoagulants being compared. In figures 4 and 5, the line of equality is closer to the mean while in 6, it is far away from the mean.

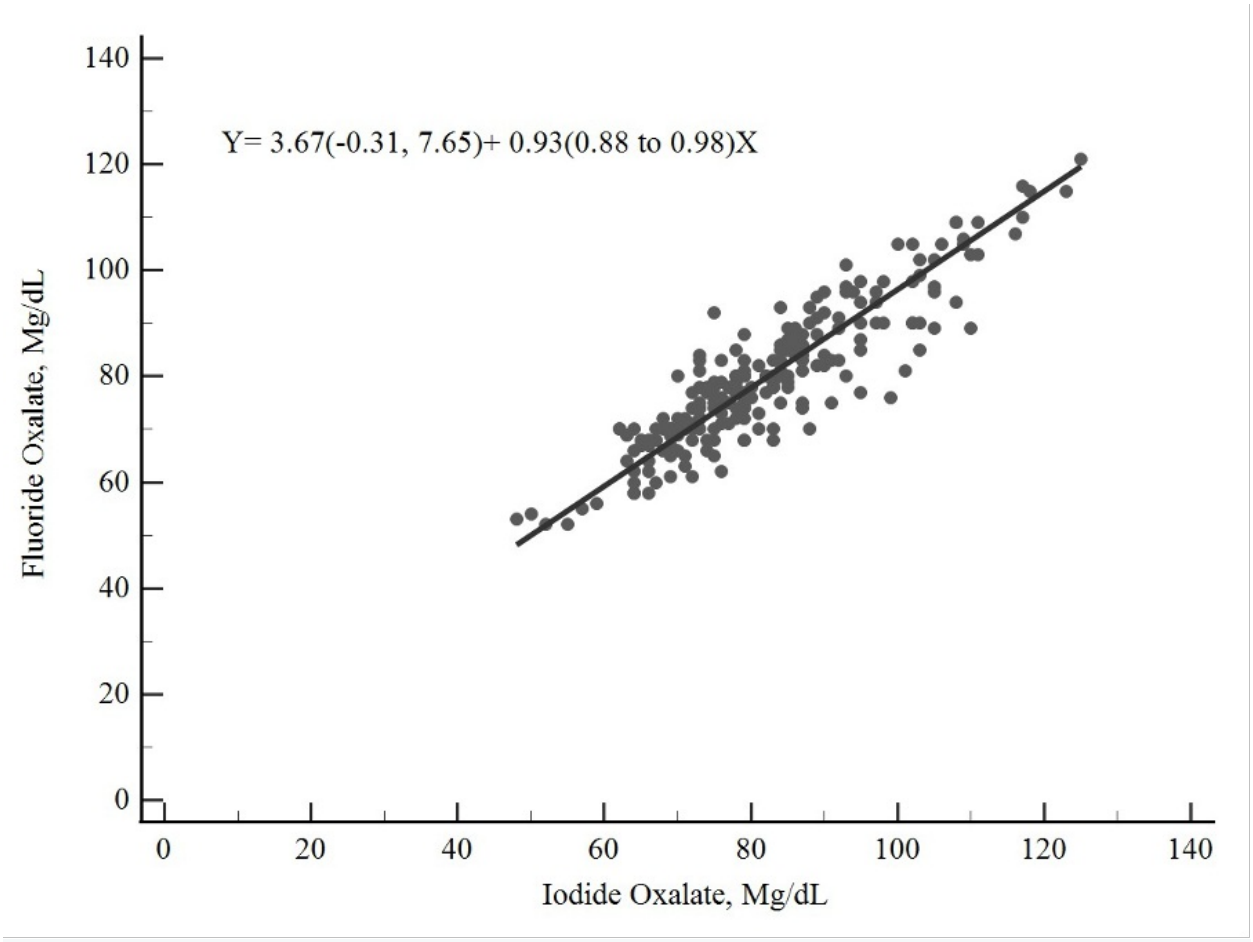


**Figure 1.** *Comparison showing Deming regression of glucose concentration measured in fluoride oxalate (reference) on the y-axis and iodide oxalate on the x-axis.For every increase in Y, there is a corresponding increase of 0.93 in X. The regression line equation is presented as Y= A (95% CI)+B (95% C1). Where 95%C1- 95% confidence interval, A-regression line intercept, B-regression line slope and Solid line -regression line.*

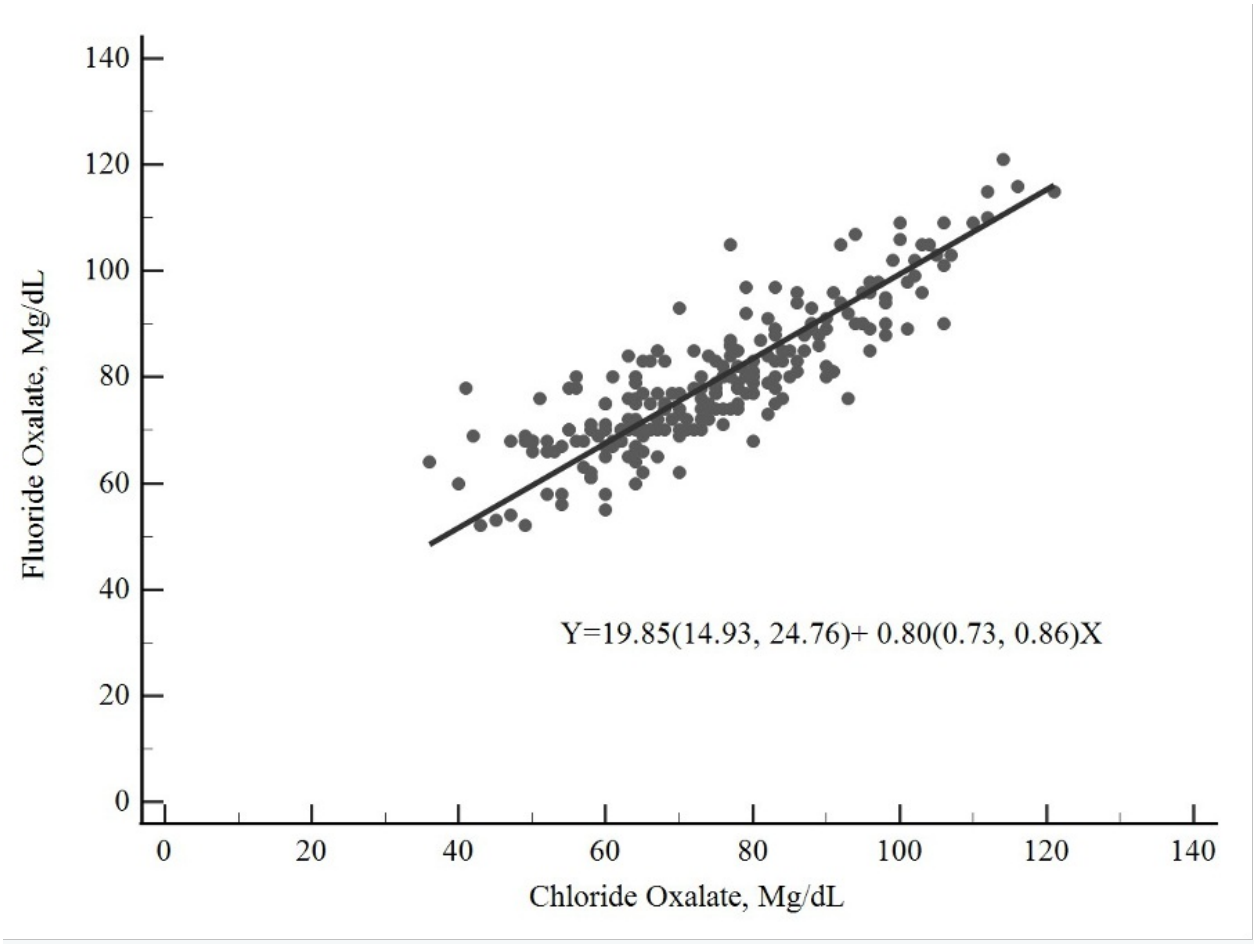


**Figure 2.** *Comparison showing Deming regression of glucose concentration measured in fluoride oxalate (reference) on the y-axis and chloride oxalate on the x-axis.For every increase in Y, there is a corresponding increase of 0.80 in X. The regression line equation is presented as Y=A(95% CI)+B(95% CI). Where 95%C1-95% confidence interval, A-regression line intercept, B-regression line slope and Solid line - regression line.*

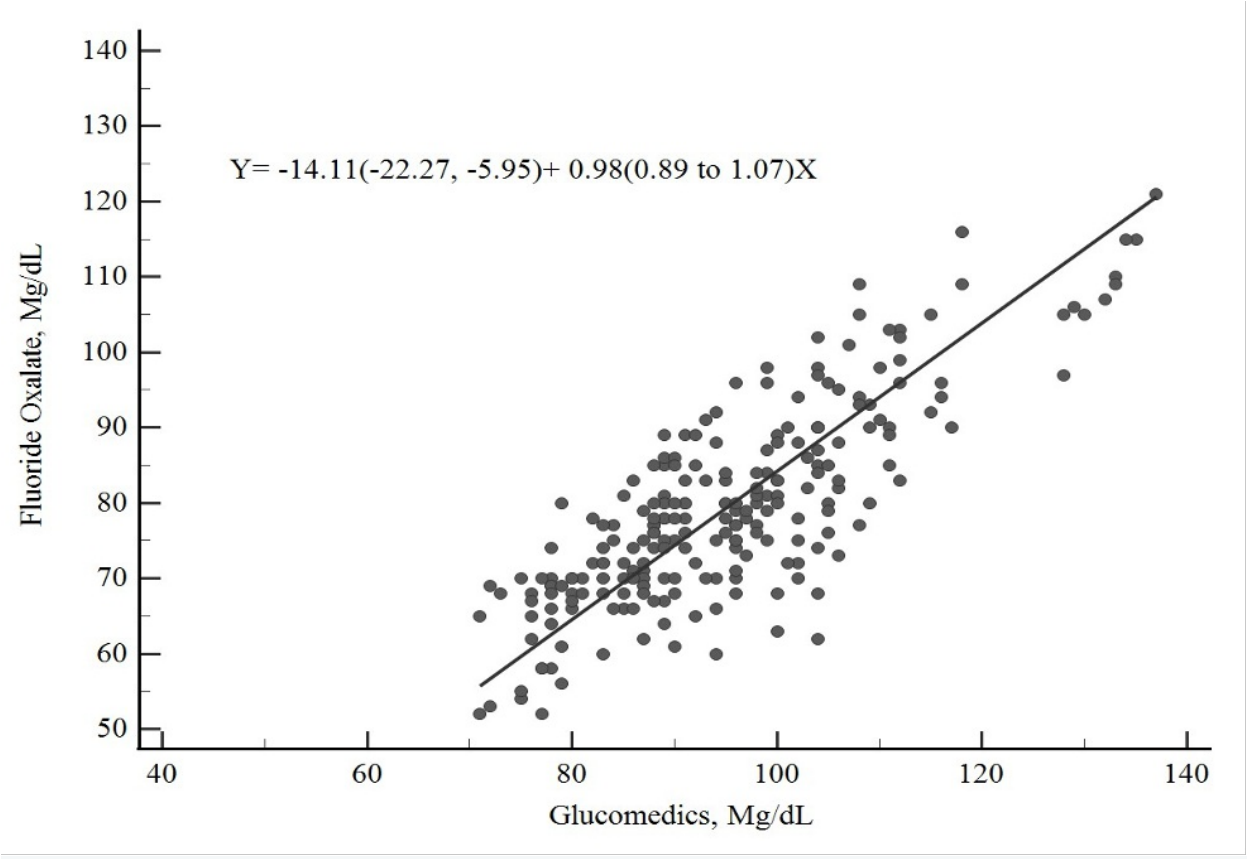


**Figure 3.** *Comparison showing Deming regression of glucose concentration measured in fluoride oxalate (reference) on the y-axis and glucomedics on the x-axis.For every decrease in Y, there is a corresponding increase of 0.98 in X. The regression line equation is presented asY=A(959CI) +B (95% CI). Where 95%C1- 95% confidence interval, A-regression line intercept, B-regression line slope and Solid line-regression line.*

**Table 2.** Pattern of Change in Glucose Concentrations (median) in the Four Tubes Measured at Different Time Points (1,2,3,4, and 24 hours)

| Tubes | Median Glucose Concentration (Range) | Z (p-value) | Bias (%) | Recommended |
|---|---|---|---|---|
| **Flouride oxalate** | | | | |
| 0-hour | 90 (83 - 101) | | | |
| 1-hour | 81 (76 - 70) | 5.847 (<0.001) | **-10.05** | |
| 2-hour | 75 (70 - 83) | 5.833 (<0.001) | **-15.75** | 2.2 |
| 4-hour | 74 (68 - 79) | 5.844 (<0.001) | **-18.28** | |
| 24-hour | 70 (66 - 78) | 5.843 (<0.001) | **-21.70** | |
| **Chloride oxalate** | | | | |
| 0-hour | 91 (83-102) | | | |
| 1-hour | 79 (73-88) | 5.770 (<0.001) | **-12.48** | |
| 2-hour | 74 (65-82) | 5.845 (<0.001) | **-18.31** | 2.2 |
| 4-hour | 64 (59-74) | 5.845 (<0.001) | **-28.02** | |
| 24-hour | 61 (53-66) | 5.843 (<0.001) | **-34.92** | |
| **Glucomedics** | | | | |
| 0-hour | 97 (89-107) | | | |
| 1-hour | 95 (88-104) | 4.853 (<0.001) | **-1.62** | |
| 2-hour | 95 (87-103) | 5.059 (<0.001) | **-3.38** | 2.2 |
| 4-hour | 94 (85-102) | 5.704 (<0.001) | **-4.93** | |
| 24-hour | 90 (81-99) | 5.851 (<0.001) | **-7.67** | |
| **Iodide oxalate** | | | | |
| 0-hour | 92 (84-103) | | | |
| 1-hour | 82 (76-91) | 5.197 (<0.001) | **-8.54** | |
| 2-hour | 79 (73-87) | 5.749 (<0.001) | **-12.01** | 2.2 |
| 4-hour | 75 (68-81) | 5.838 (<0.001) | **-18.28** | |
| 24-hour | 72 (66-79) | 5.832 (<0.001) | **-20.56** | |

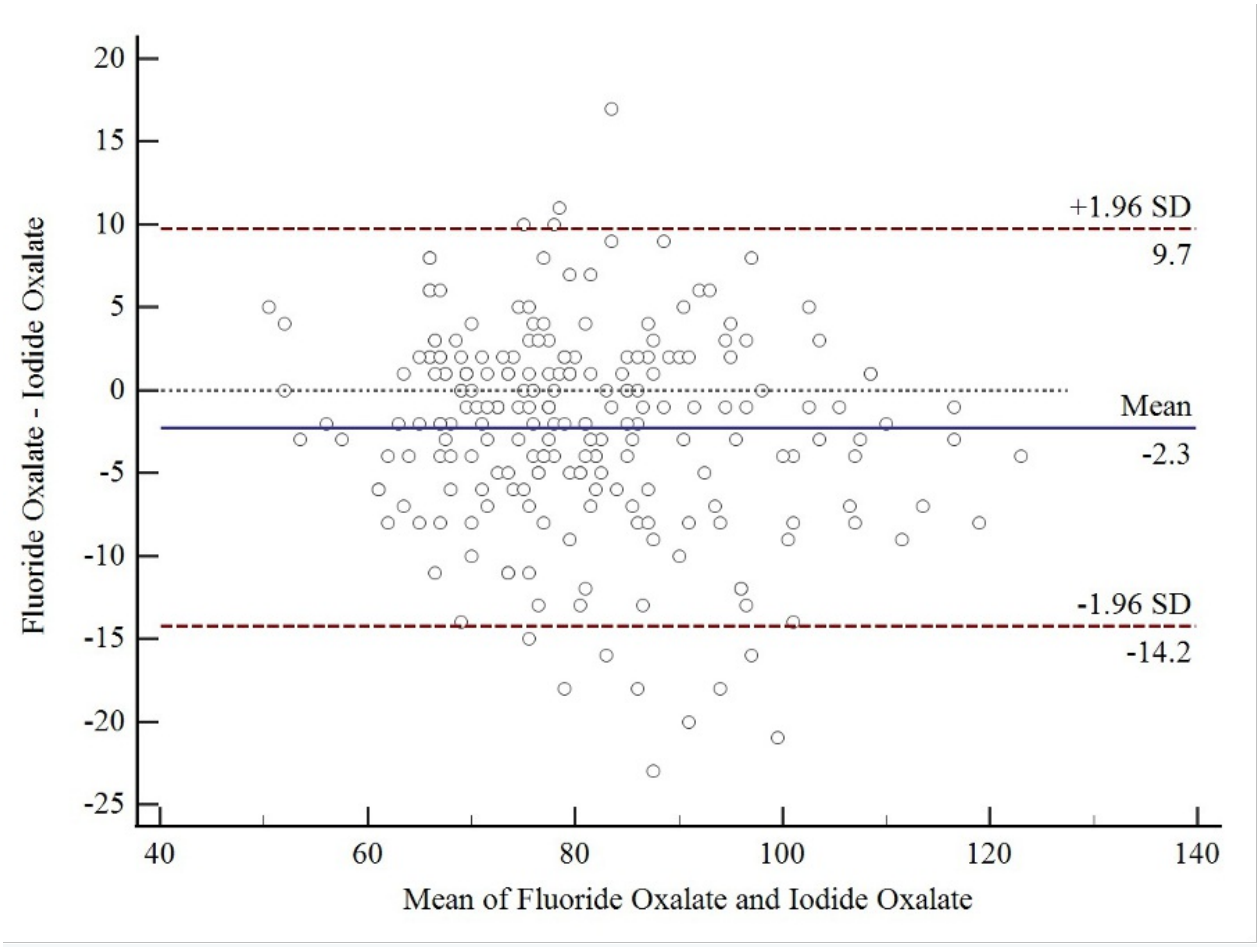


**Figure 4.** *Bland-Altman graph of difference in means to ascertain if iodide oxalate can be used as an alternative to fluoride oxalate anticoagulant. The Zero (0) on the plot represent the mean difference (bias) between the 2 different anticoagulant being compared. The closer the bias to the mean, the better the comparability. Solid line (mean)-mean difference and dashed lines - standard deviation. There is comparability between fluoride oxalate and iodide oxalate and mean falls within -2sd.*

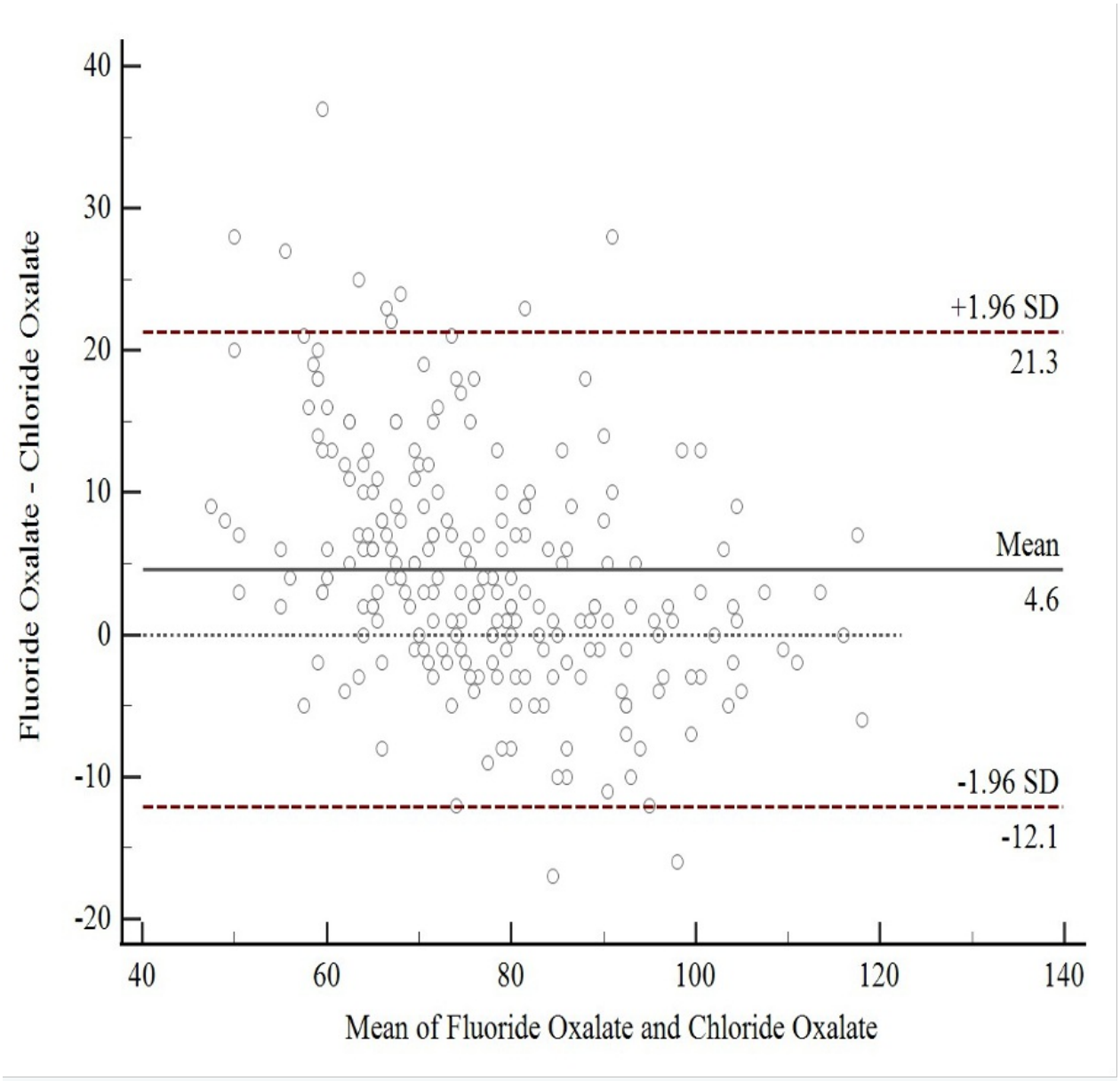


**Figure 5.** *Bland-Altman graph of difference in means to ascertain if chloride oxalate can be used as an alternative to fluoride oxalate anticoagulant. The Zero (0) on the plot represents the mean difference (bias) between the 2 different anticoagulant being compared. The closer the bias to the mean, the better the comparability. Solid line (mean)-mean difference, and dashed lines - standard deviation. There is comparability between fluoride oxalate and chloride oxalate and mean falls within 2sd*

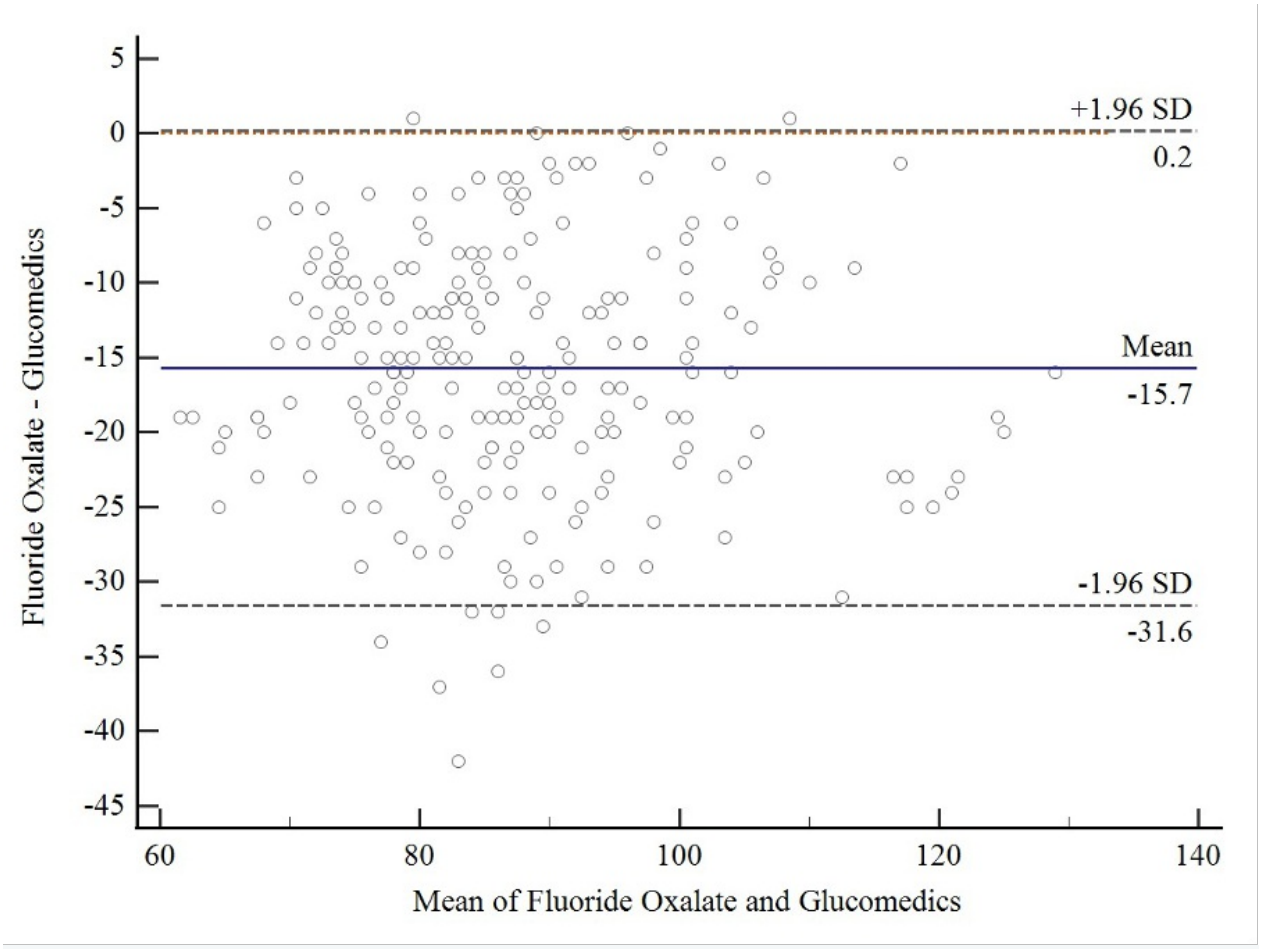


**Figure 6.** *Bland-Altman graph of difference in means to ascertain if glucomedics can be used as an alternative to fluoride oxalate anticoagulant. The Zero (0) on the plot represents the mean difference (bias) between the 2 different anticoagulant being compared. The closer the bias to the mean, the better the comparability, however the farther away from the mean, the more the disagreement. Solid line (mean)- mean difference and dashed lines - standard deviation. There is no strong comparability between fluoride oxalate and glucomedics and the mean falls on 2sd*

## Discussion

The assay of blood glucose in samples stored in anticoagulants is a regular practice in laboratory medicine. When blood samples are collected, they are stored in their native state by preserving them in different anticoagulants, though their native state is preserved, the blood glucose when assayed in different anticoagulants, at different times varies ([3][4][17]

The findings observed showed that the rate at which blood glucose concentration decreases with time varied with the type of anticoagulants. Glucose concentration in blood samples preserved in chloride oxalate decreased at a faster rate, followed by sodium fluoride and iodide oxalate while the decrease in glucose concentration in glucomedics was rather negligible.

To date, sodium fluoride oxalate (NaF/KOX) is among the most commonly used glucose inhibitors. Sodium fluoride oxalate inhibits an enzyme acting on the distal part of glycolysis (enolase), but the enzymes placed at the beginning of the glycolytic pathway remain active. The phosphorylation of the carbohydrates continues until the ATP is exhausted and this

causes consumption of glucose. So, even in the presence of NaF/KOX, stoppage of glycolysis does not occur in the first two hours after sample collection. This observation is consistent with studies reported by previous authors [9][17].

When blood is collected into bottles, the cells do not die immediately, they continue to metabolize and use up glucose as a source of energy via the glycolytic process. The lower glucose concentration values recorded for all the oxalates may be attributed to prolonged contact between red cells and blood glucose. Many studies have also reported lower values for plasma glucose using sodium fluoride oxalate as the anticoagulant [9][13][24][25]. Furthermore, previous reports have shown that the use of sodium fluoride oxalate or iodoacetate to inhibit cellular glycolytic enzymes was only partially effective because significant decrease in glucose concentration continued in the presence of either sodium fluoride oxalate or iodoacetate anticoagulants [3][14][25][26]. This indicates delayed and inefficient inhibition of glycolysis by sodium fluoride oxalate leading to false low results and over a long time, the concentration of glucose may be reduced to zero level.

Data from this study convincingly showed that a significantly higher baseline glucose concentration value was measured in glucomedics compared to NaF/KOx, iodide, and chloride oxalate tubes. This result confirms that instant glycolysis inhibition was achieved with glucomedics and this may be due to the liquid nature and combination of salts (additive) contained in glucomedics tubes which results in immediate and uniform homogenization of blood and additive (in contrast to the lyophilized additive found in NaF/KOx tubes). The finding from this study is consistent with the report of [7] who showed that glucomedics tube was the most effective in minimizing glycolysis up to 4h while NaF/KOx, EDTA, serum, and lithium heparin tubes exceeded the analytical error limit even at 1hour storage. The study by [10] using glucomedics also reported that glucose concentration was decreased by 0.3% after 2 hours and 1.2% after 24 hours at 37$^{o}$C. Consequently, a more rapid glycolysis inhibition is achieved using glucomedics preservatives. Although, [27] were the first group of researchers to show that acidification of blood was a more effective inhibitor than NaF/KOx, also Gambino et al., [10]) and Peake et al., [28]) in their research advocated for the use of combined anticoagulant tube (NaF/KOx, EDTA, citrate called glucomedics) as the tube of choice. The use of test tubes containing citric acid and sodium fluoride was recommended by the National Academy of Clinical Biochemistry (NACB) in 2011 for all those cases in which prompt centrifugation of the sample cannot be assured [8] while the use of test tubes containing only sodium fluoride should be discouraged since this only gives a delayed inhibition of glycolysis. The acidified mixture, containing citrate buffer, sodium fluoride and $Na_2$EDTA, recommended by the NACB was present in lyophilic form in test tubes already validated in numerous studies [12][29] but is no longer commercially available. This mixture is present in liquid form in other test tubes that have been shown to inhibit glycolysis effectively and promptly [30].

Furthermore, results from this study confirm that the combination of citrate buffer/NaF/EDTA in liquid form (Glucomedics tubes) is the most efficient additive in preventing glucose loss in whole blood/ plasma samples for up to 24 hours from blood collection. Results obtained also highlight that the rate at which blood glucose decreases with time varies with the specific anticoagulant, sodium fluoride oxalate, chloride oxalate, glucomedics, and iodide oxalate decreased at mean values of 15mg/dl, 17 mg/dl, 2mg/dl and 13mg/dl after 2hours respectively. With respect to the concentration of plasma glucose before storage, this suggests that storage of blood using glucomedics as an anticoagulant tends to better preserve the glucose level over a long period of time. Although at 4 hours the difference in mean was negligible when

compared with 2 hour delay for sodium fluoride oxalate and glucomedics 1 mg/dl each respectively. This also supports the possibility for sodium fluoride oxalate and glucomedics to be a better anticoagulant for long time storage of blood samples meant for glucose determinations since the glucose concentration in the blood samples stored in it tends to be comparatively more stable.

The difference for chloride and iodide oxalates was 10 mg/dl and 4mg/dl respectively. There was a further decrease at 24 hours when compared with values obtained at O hour, 1 hour, 2 hours, and 4 hours respectively. Studies conducted by Gupta and Kaur [31]) and A1Salhen *et al.,* [20] also reported similar findings, Although Chan *et al*., [14] reported that anti glycolytic action of fluoride was delayed for up to 4 hours and has little or no effect on the rate of glycolysis during the first 1-2hrs after blood was collected.

Comparing the values of glucose obtained at different time points using glucomedics, glucomedics showed a higher significant difference at 1 hour, 4 hours and 24 hours than when compared with values obtained using sodium fluoride oxalate. This implies that the antiglycolytic effect of fluoride oxalate was delayed for more than an hour before it started working, similar results were also found when compared with chloride oxalate and iodide oxalate.

The finding from this study showed that iodide oxalate, chloride oxalate, and glucomedics tubes produced comparative results with sodium fluoride oxalate and can be used when the latter is not available. However, if the samples will be stored for a longer time glucomedics is preferred while if the samples will be analyzed within hours of collection, sodium fluoride, iodide oxalate, and chloride oxalate can be used.

## Conclusion

It is obvious that irrespective of the time of collection or type of anticoagulant, the concentration of plasma glucose remained unstable after bleeding. The combination of the different anticoagulants in glucomedics has a major advantage in preserving the plasma /blood glucose when compared with sodium fluoride oxalate, chloride oxalate, and iodide oxalate.

The three different anticoagulants could be a good replacement for NaF/Kox. However, glucose values obtained using glucomedics could give a better clinically useful result than others when a delay in sample processing is inevitable. The need to consider the use of any of the anticoagulants in place of NaF/KOx is strongly recommended

Replacement of the sodium fluoride oxalate tube which is commonly used with a better and more effective glycolysis inhibitor is certainly overdue and the change in guidelines for laboratory estimation of plasma glucose is a welcome step forward.at least within our environment.

## Statements and Declarations

**Author contributions: OA** conceptualized the study, contributed to protocol development, reviewed the protocol,

supervised the study, developed the manuscript, managed manuscript submission and review; **NA** contributed to protocol development, carried out experiments and performed literature search; **AA** performed the statistical analysis and interpreted result and **CPB** reviewed and edited manuscript All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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**Data availability:** Data supporting this study are included in the article.

**Competing interests:** None declared.

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