



## **Effect of Different Anti-Coagulants on the Accuracy of Glycated Haemoglobin Results**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author FIA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DUS managed the sample collection, analysis of the study and literature searches. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Most manufacturers of glycated haemoglobin kits advocate for the use of EDTA bottles for sample collection. Other manufacturers even when using the same glycated haemoglobin assay method, advocate for the use of any of these anticoagulant: EDTA, heparin and fluoride oxalate as any of these anticoagulants for sample collection.

**Aim:** This study was therefore designed to evaluate the effect of different anticoagulants on the accuracy of glycated haemoglobin value using the same method.

**Methods:** Thirty subjects were selected by purposive sampling method and 2ml of blood was collected from each subject into sodium heparin, EDTA and fluoride oxalate bottles and stored for three days at 4°C. Fifteen subjects' samples were analysed daily for the next two days then all the samples were analysed on the third day. All samples were analysed using the boronate affinity chromatographic method by Clover.

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**Results:** The mean of the values of glycated haemoglobin of samples for each anticoagulants were about the same for the first, second and third day. The differences in the mean values for each anticoagulant were not statistically significant, indicating fairly good stability.

**Conclusion:** From this study, it could be concluded that blood sample in EDTA, fluoride oxalate and heparin bottles can be used for glycated haemoglobin estimation without affecting the accuracy of the result. These samples in these containers were found to be stable for at least three days.

*Keywords: Anti coagulants; glycated haemoglobin; chromatography; pre analytical stability.*

## 1. INTRODUCTION

Glycated haemoglobin (HbA1c) value can be used for making diagnosis and monitoring of glycaemic control in diabetes mellitus [1]. This value evaluates the mean blood glucose level over a period of 2-3 months [2] through a process of glycation of haemoglobin, which is an irreversible, non-enzymatic reaction between glucose and the amino-terminal valine (N-terminal end) of the beta chain of the haemoglobin [3].

There are a number of methods used to measure glycated haemoglobin and they include high performance liquid chromatography, immunoassay, capillary electrophoresis and boronate affinity chromatography. Most point of care devices uses either the immunoassay or boronate affinity chromatography [4]. The Clover HbA1c kit uses the boronate affinity [4].

Most glycated haemoglobin estimation kits advocate the use of EDTA specimen bottles for the collection of blood samples to be used [5], so it is, rightly or wrongly, believed to increase the accuracy of the result obtained. Other manufacturers, even when using the same analytical method, have advocated for the use of EDTA only [5]; EDTA or heparin; and EDTA or fluoride oxalate [6]. Since these anticoagulants have different mechanisms of action, it is necessary to evaluate the accuracy of the results obtained by the use of different anticoagulants. Studies have shown that the use of different anticoagulants containers for glycated haemoglobin estimation has no effect on the accuracy of the results [6]. Most times, sample for glycated haemoglobin estimation in this region have been rejected because they were not in EDTA bottles. This study was therefore designed to evaluate the effect the different anti-coagulants commonly used in clinical chemistry laboratories on the accuracy of the results of glycated haemoglobin estimation.

## 2. METHODS

This study was carried out in the clinical chemistry laboratory of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. Apparently healthy subjects who signed consent form were selected using the purposive sampling methods. For each subject 2ml of venous whole blood was collected into the EDTA, lithium heparin and fluoride oxalate bottles all manufactured for equator medics international limited in China.

Since glycated haemoglobin has high Preanalytical stability and can be stable for one week when stored at 4°C and a one year when stored at -70°C [4, 7]. The collected samples in each anticoagulant container were assayed for glycated haemoglobin same day then stored for the next two days at 4°C. Fifteen randomly selected subjects from the total subjects' stored samples were assayed daily for the next two days, while all thirty subjects samples were assayed on the third day. All measurements for glycated haemoglobin was done in duplicates and average taken.

Results from subjects were grouped under the different anticoagulants and each groups' mean taken. The difference of the means of each anticoagulant group was statistically compared and evaluated. Subjects whose samples were randomly selected for daily evaluation of glycated haemoglobin values for the three anticoagulants were also grouped accordingly and the mean taken. Subjects' haemoglobin status is important in the method adopted for glycated haemoglobin assay [8], the effects of haemoglobin concentration on the accuracy of the boronate affinity chromatographic method was not our primary target. To this effect, subject's conjunctivae, nail bed and oral mucosa was used to assess haemoglobin status.

Data analysis of variants was used to determine the difference in the values between the three

types of anticoagulant samples with graph pad prism version 6 software. A coefficient of variation was set at 95% interval and a P- value of less than 0.05 was considered significant.

### 3. RESULTS

For each subject three samples were collected into the three different anticoagulant containers. This gave a total of ninety samples and thirty each for each anticoagulant container. The mean of results in the EDTA bottles for all thirty subjects was 5.98% (SEM 0.28) while that for heparin was 5.97% (SEM 0.28). Lastly the mean value for results of samples in the fluoride oxalate bottle was 5.91% (SEM 0.29). (Table 1) The difference in the means of samples in heparin bottles and those in the EDTA bottles was not significant (P- value of 0.9997). So was the difference in the mean values of samples in fluoride oxalate bottles and EDTA bottles (p-value of 0.9997). A comparison of the means of samples in the fluoride oxalate bottle and that in the heparin bottles was also not statistically significant (p-value of 0.9831) (Table 3). The daily mean of glycated haemoglobin value for each anticoagulant was also calculated for the ten out of the thirty selected subjects. The results of the daily mean for the three anticoagulant samples measured for glycated haemoglobin were about the same (Table 3). The mean values for fluoride oxalate, EDTA and lithium heparin samples for the ten subjects for day one were 5.4%, 5.4% and 5.2% and that for day two was 5.3, 5.4 and 5.3 respectively. Lastly the mean glycated haemoglobin for day three was 5.4%, 5.4% and 5.3% for fluoride oxalate, EDTA and lithium heparin samples. (Table 3).

**Table 1. Mean results of all subjects on day 3**

	EDTA	Heparin	Flouride oxalate
Mean(%)	5.98	5.97	5.91
Std. deviation	0.88	0.87	0.90
Std. error of mean	0.28	0.28	0.29

### 4. DISCUSSION

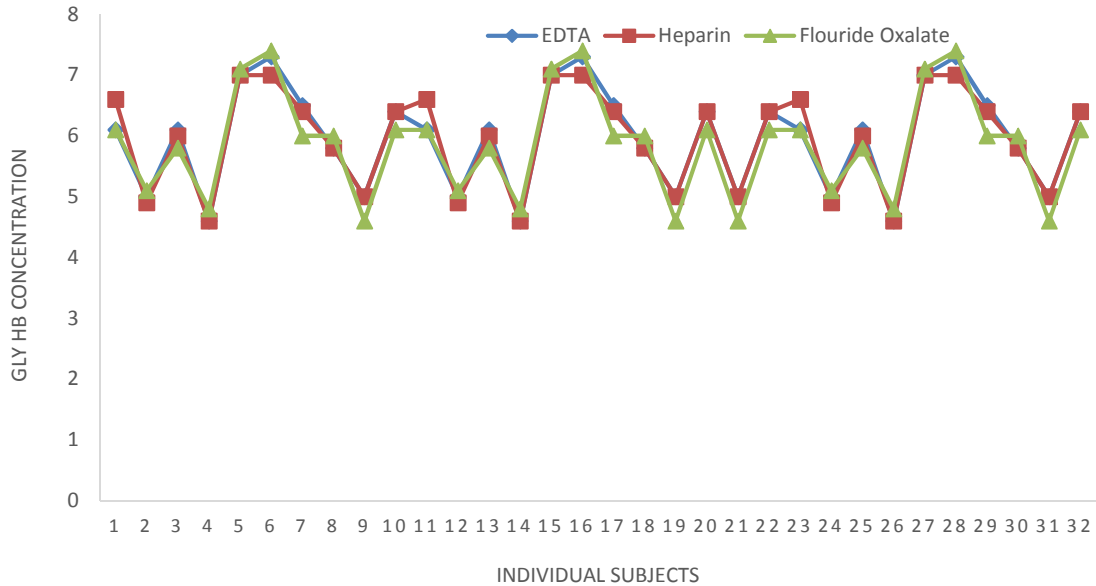
Sample tube components, such as anticoagulants, gels, and clot activators, have been recognized as a significant source of variability [9, 10]. To this effect, sample for glycated haemoglobin estimation was collected into three tubes with different anticoagulants. This was later analysed using the Boronate affinity chromatographic method by Clover. For each subject the results of glycated haemoglobin estimation from samples in the different anticoagulant bottles in this study were about the same. When all the subjects' results were grouped according to the anticoagulant (k<sub>3</sub> EDTA, lithium heparin and fluoride oxalate bottles) the difference in the means of each anticoagulant was also not statistically significant.

To this effect, it was concluded that the different anticoagulants had no effect on the accuracy of the results when using the above method. (Table 2) Results from each subject were about the same for the different anticoagulants and this trend was so for all the selected subjects as reflected in the mean results for each anticoagulant.

The difference in the means of Heparin vs EDTA, Fluoride Oxalate vs EDTA and Fluoride Oxalate vs Heparin were all found not to be statistically significant (Table 2). These finding is in keeping with a similar study done elsewhere [11], which got almost the same glycated haemoglobin values for same sample kept in different anticoagulant containers. Another study comparing two types of EDTA tubes provide no evidence of statistically significant bias in the results between K2-EDTA and K3-EDTA samples [12]. A study had samples collected into K3-EDTA, Na citrate, Na heparin and Na fluoride vials and then evaluated for glycated haemoglobin for any variation and found none [13]. This meant the mechanism of action of each anticoagulant was found not to affect the accuracy of the results so generated in this study as in other studies.

**Table 2. Comparison of means of day 3**

Tukey's multiple comparisons test	Mean Diff.	Significant?	P value
Heparin vs. EDTA	-0.01000	No	0.9997
Fluoride oxalate vs. EDTA	-0.08000	No	0.9779
Fluoride oxalate vs. Heparin	-0.07000	No	0.9831



**Fig. 1. Comparison of glycated haemoglobin in different specimen bottles from individual subjects**

The daily glycated haemoglobin mean values were about the same for all three anticoagulants and each anticoagulant daily mean was also about the same for the three days (Table 3). This was to say subject's sample was stable even in the three different anticoagulants tubes. The reproducibility of the same results for the different anticoagulant samples for the different days even as sample were stored at 4°C, was used as a measure of stability for glycated haemoglobin samples irrespective of containers. It has been established that sample for glycated haemoglobin estimation is stable for at least seven days when stored at 4°C [8] though in this study samples collected were measured daily for just three days. Therefore for this study, sample for glycated haemoglobin estimation can be said to be stable for at least three days even when using any of these anticoagulant containers.

**Table 3. Mean results of 15 subjects on Day 1 and Day 2**

Days	EDTA (mean)	Fluoride oxalate (mean)	Heparin (mean)
DAY 1	5.4%	5.4%	5.3%
DAY 2	5.4%	5.3%	5.3%

Since most haemoglobin estimations are done by few laboratories in this part of the world, samples that need to be taken to other laboratories for haemoglobin estimation can be stored for at least three day at 4°C in this area.

## 5. CONCLUSION

In conclusion, THIS study just as in other studies mentioned have shown that heparin, EDTA and fluoride oxalate tubes could be used for glycated haemoglobin estimation when using the boronate affinity chromatographic methods. It was also established that samples in this anticoagulant containers are stable for at least three days.

## 6. RECOMMEDATION

The unnecessary rejection of samples collected for estimation of glycated haemoglobin in anticoagulant tubes other than EDTA should be avoided. This will in turn reduces lost time due to sample recollection, pains of recollection, laboratory turnaround time for a better patient care.

## CONSENT

As per international standard, patient's signed consent form were selected using the purposive sampling methods.

## ETHICAL APPROVAL

As per international standard, ethical approval has been collected and preserved by the author.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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