

## Effect of Different Anticoagulants on HbA1c Values by HPLC Method at Govt Mohankumaramangalam Medical College and Hospital, Salem

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## Abstract

## Original Research Article

**Aim:** Our aim of the study is to compare HbA1c values in various Anticoagulant vacutainer tubes. **Materials and Methods:** In this study we compared HbA1c values in 30 normal individuals and 30 diabetic samples by collecting 2ml of venous blood EDTA, heparin, fluoride, citrate. We have conducted this study in our diabetology lab, GMKMCH, SALEM. Samples were analyzed on the same day between 3-5 hrs of collection in BIORAD D10 Analyser. **Results:** There was no significant differences in HbA1c values between various collection tubes. This study is important because it will reduce costs on collecting blood in separate tubes for glucose and HbA1c. This will also reduce collecting extra blood.

**Keywords:** HbA1c, Anticoagulant tubes, HPLC Method.

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### INTRODUCTION

Glycated Haemoglobin is directly related to average concentration of glucose in the blood [1]. In a normal person about 3-6 % of Hb is glycated. In a Diabetic, the percentage of HbA1c may double or even triple [2]. HbA1 is comprised of at least four distinct subfraction, HbA1a, HbA1b, HbA1c, HbA1d. HbA1c is the major component and fraction of clinical importance [3]. Glucose combines by ketamine linkage to N-terminal valine as well as lysine residues of both alpha and beta chains of Haemoglobin. The reversible adducts (pre-HbA1C) can then undergo an amadori rearrangement to stable ketamine (HbA1C)

In patients achieving their glycemic goal the ADA recommends measurement of the A1c at least twice per year, more frequent testing is warranted when glycemic control is inadequate [4]. Glycated Haemoglobin can be measured by different methods but HPLC is the standard reference method, comparable to DCCT trial. We recommend collection of HbA1c in EDTA tubes. Our aim is to compare the results of HbA1c by HPLC method in different collection tubes. This will minimize extra blood for collection and cost

on collection tube if collected the same tube for glucose.

### MATERIALS & METHODS

This study was conducted on 30 normal people from biochemistry staff and 30 diabetic patients from diabetology department, GMKMCH Salem. The study was conducted after approval from Institutional Ethical committee. The blood sample was collected on four collection tubes. EDTA, Heparin, Na Citrate, Na fluoride tubes. Two ml in all four tubes followed by proper mixing ten times. Blood was collected irrespective of timing of food. It is estimated by HPLC method Biorad D10 Analyser within 3hrs after collection.

Independent t test was used to compare the results in 4 different groups.

### RESULTS & DISCUSSION

The control group were in the age between 25-60 years. Independent t test was used to compare the HbA1c variability in different tubes among both control and cases.

**Table 1: Comparison of HBA1c values in EDTA and Fluoride tubes among controls**

	EDTA	Fluoride
Mean	5.01	5.03
SD	0.28	0.27
n	30	30
Min	4.4	4.5
Max	5.4	5.4

P value-0.8181, Confidence Interval:-0.1277 to 0.1610.

**Table 2: Comparison of HBA1c values in EDTA and Fluoride tubes among cases**

	EDTA	Fluoride
Mean	9.67	9.69
SD	2.81	2.80
n	30	30
Min	5.1	5.2
Max	15.8	15.9

P value- 0.9708, Confidence Interval:-1.426-1.479.

**Table 3: Comparison of HBA1c values in EDTA and Heparin tubes among controls**

	EDTA	Heparin
Mean	5.01	5.01
SD	0.28	0.28
n	30	30
Min	4.4	4.4
Max	5.4	5.4

P value: 0.999, CI:-1.470-1.470

**Table 4: Comparison of HBA1c values in EDTA and Heparin tubes among cases**

	EDTA	Heparin
Mean	9.67	9.67
SD	2.81	2.81
n	30	30
Min	5.1	5.1
Max	15.8	15.8

P value>0.99, CI-1.452-1.452

**Table 5: Comparison of HBA1c values in EDTA and Citrate tubes among controls**

	EDTA	Citrate
Mean	5.01	5.11
SD	0.28	0.28
n	30	30
Min	4.4	4.5
Max	5.4	5.5

P value: 0.158, CI:-0.4129-0.2480

**Table 6: Comparison of HBA1c values in EDTA and Citrate tubes among cases**

	EDTA	Citrate
Mean	9.67	9.84
SD	2.81	2.78
n	30	30
Min	5.1	5.4
Max	15.8	15.9

P value: 0.8148, CI:-1.276-1.616

From the above p values we can note that there is no significant difference between  
F luoride &EDTA tubes  
Heparin &EDTA tubes  
Citrate & EDTA tubes

This study confirms the findings of Mailankot *et al.*, [5]

This study is also in similar to Snehankar Kalika *et al.*, [6] but it is based on large group of patients and control. We have not tested the stability of HbA1c over time.

## CONCLUSION

From this study we can conclude that there is no significant difference between results in Fluoride & EDTA tubes. This study questions the need of separate container for HbA1c estimation.

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