



**ORIGINAL RESEARCH PAPER**

**Biochemistry**

**Refrigeration of Blood Sample: An effective alternative for sodium fluoride as a preservative for blood glucose samples**

**KEY WORDS:** Blood sugar, fluoride containing vacutainers /refrigerated samples in plain vacutainers

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**ABSTRACT**  
 Even with the use of fluoride it takes approximately three to four hours for these glycolytic inhibitors to become fully effective and stabilize glucose levels. If we avoid sodium fluoride as a preservative then the same samples can be used for other assays in cases when sample size must be restricted. Six samples in duplicate one with fluoride and other without fluoride are taken from diabetic patients attending our out-patient department of Heritage institute of medical sciences, varanasi. Fluoride containing sample showed 112 mg% at zero and 109 mg% at three hrs respectively (decrease of 3 mg) and refrigerated samples without fluoride, showed 108 mg% and 95 mg% respectively (decrease of 13 mg) However the decrease was not statistically significant between zero hr and 3rd hr values. Our results show that effective cooling of blood samples immediately after withdrawal can be an effective preservative than the fluoride and also we feel it should be recommended as it avoids taking two different samples if the patient wants some other tests and in cases where samples should be restricted.

**Introduction:**

The most recent guidelines of American Association for Clinical Chemistry (AACC) and American Diabetes Association (ADA) for laboratory analysis in the diagnosis and management of diabetes mellitus recommend that to minimize glycolysis, the sample tube should be placed immediately in ice water slurry and plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis.<sup>1</sup>

This is a significant change from the 2002 guidelines of AACC and ADA for glucose estimation which recommended that plasma should be separated from cells within 60 min and if that could not be done, a tube containing glycolysis inhibitor such as sodium fluoride should be used for collection of sample.<sup>2</sup>

These changes in guidelines reflect upon the ineffectiveness of sodium fluoride as an anti-glycolytic agent

Fluoride inhibits enolase, which is far downstream in the glycolytic pathway. Enzymes upstream of enolase remain active and continue to metabolize glucose until substrates are exhausted. The antiglycolytic action of fluoride is delayed for up to 4 h and has little or no effect on the rate of glycolysis during the first 1–2 h after blood is collected. Glucose levels can fall as much as 10 mg/dl during this period.<sup>3</sup>

Even with the use of fluoride it takes approximately three to four hours for these glycolytic inhibitors to become fully effective and stabilize glucose levels

**Aim & Hypothesis:**

If we avoid sodium fluoride as a preservative then the same samples can be used for other assays in cases when sample size must be restricted

Can refrigeration of blood sample be an effective alternative for sodium fluoride as a preservative for blood glucose samples?

**Materials and methods:**

Six samples in duplicate one with fluoride and other without fluoride are taken from diabetic patients attending our out-patient

department of Heritage institute of medical sciences, varanasi.

The samples with fluoride are kept at room temperature and samples without are kept at 4 degrees. In both groups blood was centrifuged and kept without separating the serum were processed every hour for three hours so as to find the difference in the glucose values.

Blood glucose in all the samples were estimated by using Glucose oxidase-peroxidase method on fully automated analyzers.<sup>4</sup>

Statistical analysis was done by using SPSS V16.0. Student's t test was performed, to compare the mean. Institutional ethical committee approval & patient informed consent were obtained.

**Results:**

Fluoride containing sample showed 112 mg% at zero and 109 mg% at three hrs respectively (decrease of 3 mg) and refrigerated samples without fluoride, showed 108 mg% and 95 mg% respectively (decrease of 13 mg) as shown in table 1.

**Table 1: Mean levels of Blood sugar in different types of sample at different time interval**

Type of Sample	Blood Sugar at 0 hr	Blood Sugar at 1 hr	Blood Sugar at 2 hr	Blood Sugar at 3 hr
Samples with fluoride	112 mg%	111 mg%	110 mg%	108 mg%
Refrigerated Samples without fluoride	108 mg%	104 mg%	100 mg%	95 mg%

However the decrease was not statistically significant between zero hr and 3rd hr values as shown in table 2.

**Table 2: p values of student's T test performed between blood sugar at 0 hr & 3 hr in different type of samples**

Type of Sample	Blood Sugar at 0 hr	Blood Sugar at 3 hr	p value
Samples with fluoride	112 mg%	108 %	>0.5
Refrigerated Samples without fluoride	108 mg%	95 mg%	>0.5

**Conclusion:**

Our results show that effective refrigeration of blood samples immediately after withdrawal can be an effective preservative than

the fluoride and also we feel it should be recommended as it avoids taking two different samples if the patient wants some other tests and in cases where samples should be restricted.

Our study proves that refrigeration of blood sample can be an effective alternative for sodium fluoride as a preservative for blood glucose samples. As the sample size was limited in our study, further studies with large sample size is required to further confirm the hypothesis.

**References:**

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