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Comparative Study of Blood Glucose Collection Methods at PSMMC

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ABSTRACT

Background: For physicians, nurses and laboratory technologists, blood plays a vital role in our work. And of the different blood components, glucose is one of those commonly measured. This may be because of its central role in metabolism and the dominance of diseases of glucose homeostasis. A problem seen in the accurate measurement of glucose is the loss of glucose because of glycolysis, during collection, transport and processing of the specimen.

Method: Specimens were taken from laboratory staff volunteers of PSMMC. Considered variables in the study were type of specimen tubes used, temperature and time elapsed before the specimen was analyzed.

Result: Results obtained showed the difference in glucose values obtained. Serum glucose measurement was higher than whole blood glucose. Whole blood glucose remained stable in refrigerated samples compared to those left at room temperature.

Conclusion: Fluoride oxalate effectively preserves glucose concentration in whole blood specimens. After the initial hour when the fluoride starts its inhibitory action, and with the specimens kept at refrigerated temperature, whole blood glucose remained stable. Temperature plays an important role in prolonging the enzymatic activity of the anticoagulant in the glycolytic pathway. Serum glucose levels can be even more reliable than whole blood specimens if the specimens are immediately spun and the serum separated from the red cells.

Keywords: Blood glucose, Blood, Serum, Glycolysis.

Abbreviations
PSMMC – Prince Sultan Military Medical City; CML & BB – Central Military Laboratory and blood Bank; CAP – College of American Pathologists; CSR – Central Specimen Reception; IFCC – International Federation of Clinical Chemistry; SST – Serum separator tubes; FO – Fluoride oxalate tubes

1. Introduction

Gene There is a continuing problem in getting the accurate glucose measurement. This is due to glycolysis, a process whereby glucose is converted to pyruvate. Glycolysis or the "splitting of sugars" occurs in the cytoplasm of the cell and during the process, energy is produced in the form of ATP. Glucose, a 6-carbon sugar is split into two molecules of 3-carbon sugars. In the 10-step process, intermediate compounds provide entry points for glycolysis. As the processes continue, more glucose will be lost. Therefore, the earlier the process is halted, the less glucose will be lost.

Several factors during the collection, transportation and handling of specimen can affect the concentration of glucose in blood. These are:

1.1 Site of Collection

There are several studies conducted [1] [2] on the difference of blood glucose values from specimens obtained from different vascular components: the veins, arteries and capillaries. Arterial blood glucose is higher than the venous blood sample. After meals venous glucose is somewhat lower than arterial and capillary glucose. Though the difference between venous and arterial glucose is minimal, capillary glucose has consistently shown higher values than venous and arterial blood samples. [3, 4]

Monitoring of the exact blood glucose lever is very important in treatment of diabetic patients as it is now accepted that insulin-based treatment regimens decrease morbidity and mortality in critically ill patients, yet strict glycemic control should be performed in a manner that minimizes the risk of hypoglycaemia [5, 6].

As a rule, blood sample collection must never be done from the arm used for infusion. There have been reported cases of increased values for blood analyses due to contamination coming from infusions made on the patient.

1.2 Specimen Type

Which is the best specimen for use in the determination of glucose: whole blood or serum? Serum has higher concentration of water and more dissolved glucose. The red cells in the whole blood samples have lower concentrations of water than serum.

Based on the 2001 recommendation of **IFCC** [7] glucose was to be reported as plasma glucose irrespective of the sample type or technology used. When done in parallel under the same conditions, results between the serum/plasma and whole blood sample will definitely be different.

1.3 Temperature

Glycolysis passes through a series of ten enzyme-catalyzed reactions. Just like most chemical reactions, the rate of the "breaking up of sugars" increases as the temperature is raised. Most enzymes increase their activity by 50 to 100 % with a 10 degree centigrade increase in temperature.

The rate of the reaction of an enzyme increases as the temperature increases, up to a maximum level; then abruptly declines with further increase of temperature. Even at moderate temperature, enzymes will no longer be active after a period of time. Enzymes stored at 5^0 C or below are generally most suitable. [4] When frozen enzymes lose their activity.

1.4 Time Frame – Collection to Analysis

One of the variables in glucose measurement is the time from sample collection until it is analyzed in the laboratory. Glucose will remain stable for 72 hours in refrigerated serum or plasma that has been centrifuged – the blood cells separated from the liquid portion of blood. If the cells are left in contact with the serum or plasma, there will be a rapid change in glucose because the red cells will continue to consume it. It has been reported that glucose in the presence of blood cells disappear at the average rate of 10 mg/dL (.55 mmol/L); but this rate increases with other factors such as glucose concentration, temperature, WBC count and other factors.

In PSMMC, glucose measurement is done in fluoride tubes (gray top tubes). For the study, the manufacturer's recommendation on proper tube inversion was strictly followed (8 inversions of 180^o) to insure proper mixing of the additive with the blood [5]. Fluoride is known to act as an enzyme inhibitor, but it takes time before fluoride becomes effective in stopping the consumption of glucose by blood cells. Therefore, with or without fluoride, the rate of losing glucose in the first hour after collection would be the same.

2. Material and Method

2.1 Subject

Blood specimens from 30 volunteers were used for this study. A total of four tubes were collected from the volunteers: 1- SST and 3- FO tubes. Collection of samples was timed for regular check-up of for routine chemistry and glucose testing.

Table 1: Summary table for the specimen tubes collected from each volunteer.

SST	**FO tube # 1**	**FO tube # 2**			**FO tube # 3**		
Centrifuged 20 mins. after collection							
Cells separated from serum		Kept at room temperature (27^oC)			Kept at refrigerated temperature ($2\text{-}8^0C$)		
Analysis done and recorded	Analysis done and recorded as initial glucose reading	Analysis done after 1 hour	Analysis done after 2 hours	Analysis done after 3 hours	Analysis done after 1 hour	Analysis done after 2 hours	Analysis done after 3 hours

2.2 Experimental Design

A. SST versus FO Tubes

The SST tube and one FO tube from each volunteer was simultaneously centrifuged 15-20 mins. after collection and analyzed for glucose. The two extra FO tubes were used for the second part of the study. Serum was separated from the red cells. Results are logged in Table 2.

Table 2: Glucose results obtained for the thirty volunteers; using FO and SST tubes. The %change or difference is shown in the last column.

Sample No.	FO Tube	SST	% Change
	Centrifuged after 20 mins.	Centrifuged after 20 mins.	Yellow/Gray
1	18.62	18.91	1.53
2	12.93	13.08	1.15
3	17.31	17.53	1.25
4	6.62	6.68	0.90
5	4.55	4.58	0.66
6	3.34	3.36	0.60
7	10.58	10.68	0.94
8	7.89	7.97	1.00
9	8.64	8.72	0.92
10	11.61	11.74	1.11
11	4.2	4.23	0.71
12	22.26	22.96	1.57
13	13.86	14.05	1.35
14	9.91	10.01	1.00
15	16.46	16.65	1.14
16	5.42	5.47	0.91
17	17.89	18.14	1.38
18	19.64	19.95	1.55
19	8.74	8.82	0.91
20	11.01	11.13	1.08
21	3.99	4.01	0.50
22	6.78	6.82	0.59
23	10.11	10.22	1.08
24	18.74	19.04	1.58
25	7.84	7.92	1.01
26	3.72	3.74	0.53
27	4.11	4.13	0.48
28	4.92	4.96	0.81
29	5.94	5.99	0.83
30	6.91	6.95	0.58
The average percent change for all 30 samples ----------0.99			

B. Fluoride oxalate tubes in refrigerated and room temperature
Samples from 30 laboratory staff volunteers were used. Each volunteer had 3 fluoride oxalate tubes for analysis. Gray tube # 1 was used for the initial reading of glucose in experimental design A. Gray top tube # 2 was kept at refrigerated temperature (30C) and was analyzed after 1, 2 and 3 hours. Gray top tube # 3 was kept at room temperature (270C) and was analyzed after 1, 2 and 3 hours. Results obtained were logged in Table 3.

Table 3: Tabulated values of glucose, recorded 1, 2 and 3 hours after collection.

Sample No.	Gray top # 1	Refrigerated (Gray top # 2)			Room Temperature (Gray top # 3)		
	After collection	*1 hr.*	*2 hrs.*	*3 hrs.*	*1 hr.*	*2 hrs.*	*3 hrs.*
1	18.62	18.62	18.6	18.59	18.53	18.44	18.31
2	12.93	12.95	12.94	12.94	12.84	12.76	12.63
3	17.31	17.34	17.32	17.32	17.27	17.12	17.06
4	6.62	6.65	6.61	6.6	6.54	6.48	6.43
5	4.55	4.56	4.53	4.51	4.5	4.45	4.42
6	3.34	3.33	3.32	3.32	3.3	3.29	3.28
7	10.58	10.59	10.59	10.59	10.55	10.5	10.47
8	7.89	7.89	7.88	7.87	7.85	7.8	7.75
9	8.64	8.64	8.63	8.62	8.6	8.51	8.42
10	11.61	11.61	11.6	11.59	11.6	11.54	11.42
11	4.2	4.22	4.22	4.21	4.18	4.12	4.08

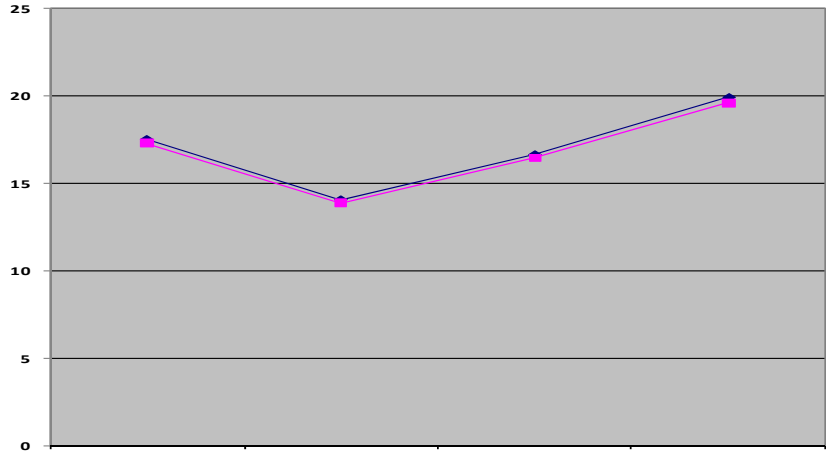
12	22.6	22.61	22.62	22.6	22.4	22.35	22.21
13	13.86	13.85	13.85	13.84	13.84	13.75	13.62
14	9.91	9.93	9.92	9.9	9.89	9.81	9.74
15	16.46	16.47	16.45	16.44	16.4	16.35	16.25
16	5.42	5.42	5.41	5.4	5.4	5.39	5.37
17	17.89	17.88	17.87	17.84	17.84	17.8	17.74
18	19.64	19.65	19.64	19.63	19.6	19.52	19.41
19	8.74	8.75	8.74	8.74	8.7	8.65	8.52
20	11.01	11.03	11.01	10.98	10.99	10.85	10.74
21	3.99	3.99	3.98	3.97	3.96	3.92	3.9
22	6.78	6.78	6.77	6.76	6.76	6.74	6.73
23	10.11	10.12	10.12	10.11	10.09	9.97	9.84
24	18.74	18.76	18.74	18.71	18.64	18.55	18.35
25	7.84	7.85	7.82	7.78	7.81	7.79	7.74
26	3.72	3.72	3.71	3.71	3.7	3.67	3.65
27	4.11	4.12	4.12	4.11	4.08	4.05	4.02
28	4.92	4.91	4.91	4.9	4.9	4.89	4.82
29	5.94	5.96	5.93	5.93	5.91	5.86	5.81
30	6.91	6.91	6.9	6.9	6.88	6.82	6.78
Mean	**10.16**	**10.17**	**10.16**	**10.15**	**10.12**	**10.06**	**9.98**
SD	**5.61**	**5.61**	**5.61**	**5.61**	**5.58**	**5.57**	**5.53**

2.3 Analytical Procedures

Glucose was analyzed on Roche COBAS 8000 modular analyzer. The test principle is an enzymatic reference method with hexokinase. This method is the most specific method for measuring glucose in serum or plasma, wherein hexokinase plus ATP glucose 6-phosphate is then reacted with NADP and glucose 6-phosphate dehydrogenase to form NADP which is measured spectrophotometrically.

2.4 Statistical Analysis

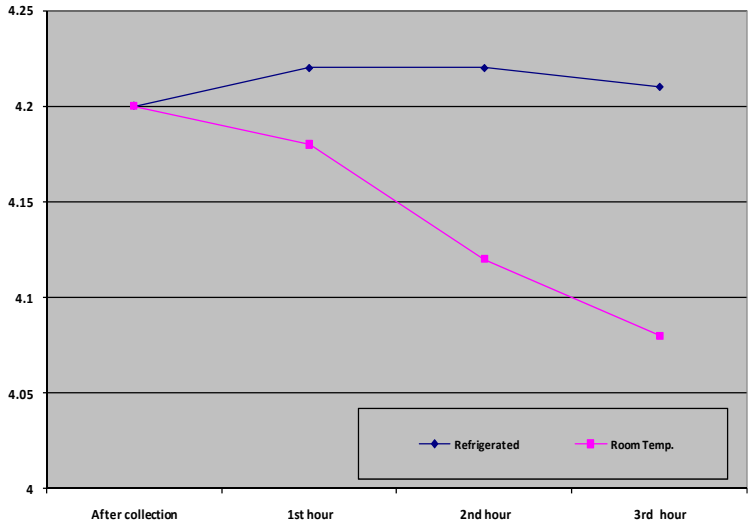
A. Blood glucose levels in SST tubes which were centrifuged 20 minutes after collection yielded higher glucose values. The average % difference for all 30 samples is 9.9. To get the glucose serum/plasma value from whole blood glucose figures, it is multiplied by 1.11. This constant factor is according to the recommendation of IFCC [7]; and based on the relationship of whole blood or serum/plasma glucose at normal hematocrit. With lower hematocrit, the smaller the difference; the higher the hematocrit the bigger the difference between the glucose levels in the two sample types.



Graph 1: A graphical representation of glucose values obtained from SST and FO tubes.

B. In the first hour after collection, glucose values obtained for both refrigerated and room temperature samples are almost the same. After the 1st hour, there is a noticeable drop in glucose level for samples left at room temperature. Refrigerated samples somehow slowed down in losing the glucose after the 1st, 2nd and 3rd hour compared with samples left at room temperature.

A graphic illustration of the results was made to show the drop in glucose readings from the initial results obtained 20 minutes after collection and the results obtained at one, two and three hours after specimen collection.



Graph 2: Graphical representation of the drop in glucose value with temperature and time as variable.

3. Results

Based on the study done, and with all information logged and presented in the tables above, results of the study can be summarized as follows:

Serum blood glucose can yield a more accurate measurement if immediate centrifugation is done to separate the red cells from the serum. It takes time, almost an hour, before fluoride start acting as an inhibitor. In the 1st hour after collection, with or without fluoride the glucose value will be the same. Samples sometimes reach up to 4 hrs. before it is analyzed. In this case glucose values obtained are no longer reliable as a true measure of the glucose level.

Enzymatic activity slows down when at lower temperature. Fluoride samples kept in the refrigerator will yield a more reliable glucose value compared with the sample left at room temperature that is considering all other parameters are the same.

In serum or plasma samples, if cells are separated immediately, and the sample is kept in refrigerated temperature, glucose will remain stable for 72 hrs.

4. Discussion

Results of glucose testing are likely to change frequently or suddenly due to the numerous variables that can affect the process. In a featured article in CAP Today on May 2005 [8], it was discussed that there is marked variability in both biologic and analytic glucose testing. Greater variability is encountered during sample collection and handling prior to its arrival in the laboratory. The study conducted was limited to comparison of glucose values with regard to sample type, effect of temperature and the length of time from sample collection until analysis. Fluoride samples must be analyzed within an hour after collection. If not analyzed, separation of cells through centrifugation or keeping the plasma sample at 4 ^{o}C will help preserve the glucose [10, 11]. With the current set up of most routine laboratories, the best way to eliminate glucose loss would be to send glucose samples immediately to the laboratory for analysis, preferably within the 1st hour after collection. Sending the specimens in refrigerated temperature will be an added factor to have a more suitable specimen for glucose analysis.

Future plans for improvements in the collection and handling for glucose samples can be introduced. A further follow up study in using SST tubes for glucose analysis instead of fluoride tubes is recommended [11,12,13]. From remote collection sites of the laboratory, centrifuge machines can be installed so that specimens can be centrifuged as soon as possible, before it is delivered to the laboratory for analysis. Self-monitoring can play vital role in monitoring diabetic patients glucose and can prevent patients form metabolic shock but exact glucose level can be monitor after recommend precaution for collection of blood collections [14-16].

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