Comparison of Glucose Determinations on Blood Samples Collected in Three Types of Tubes

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Abstract. Because of the metabolism of serum glucose in collection tubes containing blood samples, serum glucose levels may be found to decrease over time. Several types of collection tubes have been designed to, at least partially, block glucose metabolism by red blood cells in blood collection tubes that may not be analyzed immediately after blood collection. These include red-top collection tubes with serum separator, grey-top tubes with a fluoride glycolysis inhibitor, and heparin-containing green-top tubes which prevent clot formation. As part of a quality assurance project, we investigated whether glucose levels differed in the three tube types from each of 18 volunteers on a prolonged standing of 4 hours. We then determined the glucose concentrations of all three tubes from each of the 18 volunteers. We used refrigerated samples over a five-day period to determine if the initial values were reproducible. Surprisingly, after standing for four hours at room temperature, we found that the glucose levels in the three tubes from each volunteer were statistically indistinguishable from one another using the two-tailed paired t-test. Also, a linear regression analysis showed that the values of glucose for the three pairs of two tube types were closely correlated with one another, with correlation coefficients of >0.97, slopes close to 1, and Y-intercepts close to 0. These results suggest that blood collection in any of these tubes will render similar values for serum glucose even after standing for four hours. The tubes were then refrigerated at 4°C and re-analyzed after another six hours and then once per day for the next four days. Beginning at the first day at the six-hour determination, the glucose levels in the red- and grey-top tubes were statistically indistinguishable from one another but not in the red- and green-top tubes and in the grey- and green-top tubes. This was due to a steady decrease in the glucose levels in the green-top tubes. The glucose levels in the red- and grey-top tubes from each volunteer remained constant over the five-day period so that the coefficients of variation (CV) were low. In contrast, due to the decrease of glucose levels in the green-top tubes, the CVs for repeated glucose determinations in these tubes were high. Interestingly, a regression analysis of the glucose values for all three sets of paired tubes showed high (0.97) correlation coefficients and slopes close to 1. However, a regression analysis of the glucose values in the red- and green-top and grey- and green-top tubes at day five showed Y-intercepts of about -32 suggesting that there is a constant decrease of glucose in the green-top tubes that amounts to approximately 6 mg/dL per day over five days. These results suggest that red-top tubes with serum separator or grey-top tubes with a fluoride glycolysis inhibitor may be used for reproducible glucose determinations.

Introduction

Sensitive and stable analytical methods for quantitation of serum glucose levels have been developed which include the hexokinase-glucose-6-phosphate dehydrogenase and glucose oxidase assays [1]. The major problem with glucose determinations is the pre-analytical factor of the type of tubes used for blood collection.

Since red blood cells metabolize glucose via glycolysis, their presence can lower glucose concentration in whole blood to as much as 7 mg/dL per hour [2]. To prevent this occurrence, blood can be collected into red-top tubes with a serum separator gel that sequesters the cells from plasma/serum [3]. In addition, grey-top tubes can be employed that contain fluoride ions which inhibit glycolysis. To prevent clot formation that can distort plasma glucose determinations, green-top tubes with the anti-coagulant, heparin, have been employed for use in glucose assays. The question then arises as to which
tube is the best for these assays and which allows for the most reproducible determinations of glucose over time. This latter question is important in view of requests for repeat determinations of glucose on previously assayed samples. While such requests are infrequent, their accuracy must be maintained so that reliable repeat determinations can be made when necessary.

In an early study [4] of the effects of inhibitors of glycolysis (fluoride and thymol) on serum glucose determinations, blood samples from over 200 patients were collected in tubes containing the glycolysis inhibitors and in tubes without these inhibitors or preservatives. Significant differences in glucose levels between the two tubes for most patients were found when the tubes were analyzed after a one hour waiting period at room temperature. For most of the paired samples, glucose levels were significantly lower in the untreated tubes with no inhibitors although in some samples the glucose levels in the untreated tubes were significantly higher than in the treated tubes. The conclusion of this study was that, if blood is collected in untreated tubes, glucose determinations must be made immediately.

In a subsequent study [5] where the paired tubes contained either fluoride or heparin, it was found that after a period of about one hour, whole blood glucose was significantly higher in the fluoride tubes as compared to that in the heparin tubes. A similar result was found for the serum (although the decreases in glucose concentration in the heparin tubes compared with the decrease in the fluoride tubes was less than that found for whole blood). It was concluded that fluoride exerts its effects slowly, requiring about four hours to take full inhibitory effect. This finding has been confirmed more recently [6] and is because fluoride blocks glycolysis at later steps, i.e., inhibition of enolase, so that even though lactate production is still strongly inhibited, glucose is still metabolized until a significant depletion of ATP occurs. In the study in reference 4, it was found that use of either tube type was valid if glucose determinations are performed within an hour after blood collection. In this study, irrespective of the type of tube used for blood collection, a significant decrease in glucose levels was observed in the first hour.

All of these studies implied that if blood samples are allowed to stand for one or more hours, there is the possibility that serum glucose levels will differ depending on the tube in which the blood sample was collected. This type of consideration prompted the Endocrinology Service at our medical center to inquire whether collection tubes could affect serum glucose levels and if so, which tube(s) would be the best for serum glucose determinations. In addition, the question arose as to how stable glucose values are in tubes stored in a refrigerator over time in each of these different tube types. This question is important because, although infrequently, repeat determinations of serum glucose are requested.

As part of a quality assurance investigation to answer these questions we instituted a study in which we collected blood samples for glucose assays from eighteen subjects. Each sample was collected into three tubes, red-top with serum separator, grey-top with sodium fluoride, and green-top with heparin. We compared the results from the samples in the three different tube types which were obtained after an estimated maximum amount of time that should be allowed between blood collection and serum analysis, i.e., 4 hours. We then assayed these same tubes over a five-day period to determine the stability of glucose levels in each of the three different tubes of each subject. In pursuing the first objective, we'd like to note that it was not our objective to determine the initial glucose values which may have been significantly higher than those found after the four hour standing period. Rather, we sought to determine whether significant differences occur in the glucose concentrations among the three different tubes given a prolonged standing time. We then explored whether glucose values changed over time in each tube on which the initial determinations were made when the tubes were stored at 4°C.

Methods

This study was undertaken originally as part of a quality assurance project to determine what blood tubes are best suited to give accurate and stable serum/plasma glucose levels, i.e., which tube type allowed for the highest reproducibility when assayed during storage. Blood samples were collected from eighteen healthy volunteers (although one is diabetic) from the laboratory staff and included several Pathology residents into three tubes (Becton-Dickinson, Franklin Lakes, NJ): and placed into three tube types: red-top with serum separator; grey-top with fluoride; and green top with heparin. The age
range of the volunteers was 30-59, 13 females and five males. The group included 7 caucasian, 1 Pakastani, 5 African-American, 2 oriental, 1 Indian, and 2 hispanic individuals. The samples were then allowed to stand at room temperature for four hours after which they were centrifuged and placed on the autoanalyzer (see below) so that the serum layers could be assayed for glucose levels. All tubes were then placed in the sample refrigerator at 4°C and then re-assayed after 6 hours. The samples, stored at 4°C, were then assayed once per day for the next four days. All glucose determinations were carried out on the Siemens (Tarrytown, NY) Advia 1800 autoanalyzer using the hexokinase method [1]. In all subsequent determinations after the first analysis, prior to assay, the grey-and green-top tubes were subjected to centrifugation followed by placement on the analyzer for the serum glucose assay. Since the serum separator in the red-top tubes completely separates the serum from cells after a single centrifugation, these tubes were analyzed directly without centrifugation on all red-top tubes after the initial glucose assays at 4 hours. The range for glucose values was 75-120 mg/dL, i.e., a 45 mg/dL range, for 17 of the 18 volunteers; one individual is diabetic and was found to have a serum glucose value of 400 mg/dL.

At each time point, all glucose values obtained for each tube for each subject were compared using the two-tailed paired t-test to determine if the values were statistically significantly different. In this procedure, red-top tube results were compared with grey-top tube results; red-top tube results were compared with green-top tube results and grey-top tube results were compared with green-top tube results. P values of <0.05 were considered to show statistically significant differences between two data sets; p values ≥ 0.05 were considered as indicating that there is no statistically significant difference between the two data sets. Coefficients of variation for glucose values for each tube of each subject were computed to determine the precision for glucose level determinations in each tube type. Using the program EP Evaluator (Data Innovations, Burlington, VT), a linear regression analysis was performed for the glucose concentrations of all samples for the three sets of two tube types at each time when these glucose concentrations were determined, i.e., grey-top vs red-top, green-top vs red-top and green-top vs grey-top. Correlation coefficients ≥ 0.9 were considered to indicate that the points fit the computed best-fit straight line through them.

### Table 1. P-Values* for correlation of glucose values between different tube types

<table>
<thead>
<tr>
<th>Time</th>
<th>Red-Green</th>
<th>Red-Grey</th>
<th>Grey-Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1-Initial</td>
<td>0.007</td>
<td>0.026</td>
<td>0.181</td>
</tr>
<tr>
<td>Day 1-initial+6 hr</td>
<td>0.002</td>
<td>0.774</td>
<td>0.317</td>
</tr>
<tr>
<td>Day 2</td>
<td>3.19X10^-8</td>
<td>0.069</td>
<td>2.12X10^-8</td>
</tr>
<tr>
<td>Day 3</td>
<td>7.44X10^-10</td>
<td>0.893</td>
<td>2.00X10^-9</td>
</tr>
<tr>
<td>Day 4</td>
<td>1.81X10^-12</td>
<td>0.914</td>
<td>2.97X10^-12</td>
</tr>
<tr>
<td>Day 5</td>
<td>3.26X10^-10</td>
<td>0.456</td>
<td>5.35X10^-7</td>
</tr>
</tbody>
</table>

*P values <0.05 are considered significant

### Table 2. Correlation coefficient, slope and intercept between different tube types

<table>
<thead>
<tr>
<th>Time</th>
<th>Tubes Correlated</th>
<th>R(Correlation Coefficient)</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Green-Red</td>
<td>0.9995</td>
<td>1.004</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Grey-Red</td>
<td>0.9997</td>
<td>0.995</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Green-Grey</td>
<td>0.9997</td>
<td>0.992</td>
<td>-0.4</td>
</tr>
<tr>
<td>Day 5</td>
<td>Green-Red</td>
<td>0.9882</td>
<td>0.988</td>
<td>-32.6</td>
</tr>
<tr>
<td></td>
<td>Grey-Red</td>
<td>0.9960</td>
<td>0.998</td>
<td>-1.0</td>
</tr>
<tr>
<td></td>
<td>Green-Grey</td>
<td>0.9746</td>
<td>1.011</td>
<td>-38.1</td>
</tr>
</tbody>
</table>

### Table 3. Mean, minimum and maximum CVs for each type of tubes for the 18 patients in this study

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Mean (%)</th>
<th>Standard Deviation</th>
<th>Minimum CV (%)</th>
<th>Maximum CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Top</td>
<td>1.0</td>
<td>±0.4</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Green Top</td>
<td>16.0</td>
<td>±6.8</td>
<td>3.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Grey Top</td>
<td>3.0</td>
<td>±3.5</td>
<td>1.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>
Comparison of glucose determinations on blood sample

Results

Correspondance of glucose values in the three types of tubes. Figure 1 shows the comparison of values between the different tube types at all six time points. Initially, on Day 1 (D-1 in the figure), the values in the three tubes for each blood sample seem to be close to one another. As shown in Table 1, the two-tailed paired t-test revealed that the only set of tubes where the values are statistically indistinguishable from one another, i.e., p values of ≥0.05, are for grey- and green-top tubes. However, the p value of 0.026 for the values of glucose in the red- and grey-top tubes suggests that there is borderline non-distinguishability between these values, i.e. that they may be the same.

Clearly at 6 hours (D-1+6h in the figure), the values of glucose in the red- and grey-top tubes are statistically insignificantly different from one another (p value=0.774) as are the values in the grey- and green-top tubes, as shown in Table 1. Examination of entries 3-6 in Table 1 reveals that, beginning with day 2 and extending through day 5, the only glucose values that are statistically insignificantly different are those in the red- and grey-top tubes. In accordance with this result, inspection of the graphs in Figures 1 shows that from day 2-day 5, the values for red- and grey-top tubes are, for the most part, close to one another while the values for the green-top tubes are significantly lower for most samples.

Linear regression plots for glucose determinations in the three tube types. Figures 2 show the regression plots for the three pairs of tubes tested for day 1 and day 5. As can be seen in this figure, the correlation in each case is excellent. On day 1 the correlations between results for all three paired tubes were close (see upper Figure 2 and Table 2). As shown in Table 2, the correlation coefficient (R) was ≥0.99 for all three correlations. The slopes for all three plots were close to 1.0, and the intercepts were close to 0. Importantly, the single diabetic value of
approximately 400 mg/dL occurred on the best-fit straight line although it was a high value that fell above the next highest value of 120 mg/dL.

On day 5, the R values were ≥ 0.97 for all three regression plots, and the slopes were close 1. The intercept for the grey-vs red-top tubes remained close to 0. However, on day 5, where the statistical correlation between green tube and red or grey tube results was poor, all regression plots for green tubes (vs red- or grey-top tubes) have intercepts of -32 mg/dL, i.e., green-top tube glucose values can be computed on day 5 from red- or grey-top tube values by subtracting 32 from the latter. Thus the glucose concentration in the green-top tubes decreases about 32 mg/dL over the five day period relative to the corresponding value in the red-and grey-top tubes, or a decrease of approximately 6 mg/dL glucose per day. The absence of statistical correlation (low p values) of the green-top tube with the red- and grey-top tubes on day 5 is due to this constant subtraction factor.

Strong reproducibility of glucose values in red- and grey-top but not green-top tubes. Inspection of the results in Figures 1 suggest that the glucose concentrations in the red- and grey-top tubes are highly reproducible, including the diabetic high value of around 400 mg/dL. More quantitatively, Table 3 summarizes the results for the coefficients of variation (CV) for each subject in each tube type. Clearly, the lowest mean CV value, 1 percent, is that for the red-top tubes and is one-third of that for the grey-top tube and one sixteenth of the mean CV for the green-top tube. The maximum CV for this tube type is 3 percent while the maximum for the grey-top tube is 14 percent. The latter value was for one sample which was first taken on day 2 for the other seventeen samples. The glucose levels were 92, 94 and 93 mg/dL for days 2-3 and 69 mg/dL on day 5. If this sample is omitted from the computation of the CVs, then maximal CV becomes 9 percent. Both values for the grey-top tubes are significantly lower than that for the green-top tubes, which is 32 percent. Overall, these results suggest that red-top tubes with serum separator and grey-top tubes yield reproducible glucose values while green-top tubes do not. The most reproducible results are obtained in red-top tubes with serum separator.

Discussion

We had two objectives in this study: determine whether serum glucose values in the three different tube types (red-top tubes with serum separator, grey-top tubes with fluoride glycolysis inhibitor, and green-top tubes with heparin anti-coagulant) were the same when blood samples were allowed to stand for four hours and determine how stable the subsequent glucose levels are in these tubes over time. This study differed from other previous studies because it did not aliquot blood samples for different time points; rather, we allowed each tube to stand for 4 hours and then assayed the tubes for their respective glucose concentrations. We then tracked the glucose level in each tube over the course of five days.

Effect of Tube Type on Serum Glucose levels.

Results of Past Studies Suggest Collection Tube Type Influences Glucose Values If Analysis Is Performed After 1 Hour. Past studies have found that serum glucose levels in tubes containing fluoride, an inhibitor of glycolysis, are significantly higher than in heparin-containing tubes if the tubes stand for an hour prior to analysis [4,5]. These results extend through four hours at which time the fluoride is thought to have exerted its maximal inhibitory effect on the glucose metabolism in red cells [5,6]. The overall conclusion from these studies was that agreement of glucose values between the two sets of tubes is obtained if glucose determinations are made within one hour after sample collection.

Unless tubes are specified as “stat,” i.e., analysis must be performed within one hour after sample collection, tubes often stand for longer periods of time than 1 hour. Since stat analysis applies to about one-third of the samples received in the laboratory at our medical center, about two-thirds of these samples are analyzed in time periods greater than one hour. This opens the possibility that glucose determinations for the majority of samples received in the laboratory may be affected by the tube in which they were analyzed.

To determine whether serum glucose levels were affected by the tube in which they were collected, we allowed the three types of blood collection tubes to stand at room temperature for 4 hours, the maximum time that tubes should be allowed to stand prior to their analysis.

Our Results Suggest that Glucose Values Are the Same in the Three Tube Types Even If Allowed To Stand at Room Temperature for 4 Hours. In contradistinction to the results in past studies, we find
Comparison of glucose determinations on blood sample

that the serum glucose levels in all three tube types for each of the 18 volunteers are statistically insignificantly different from one another at the four hour time point (Table 1 and Figure 1). In Figure 1, the actual glucose values can be seen to correspond to one another closely at the first (4 hour) time point. This conclusion is reinforced by our finding that the glucose values from the three tubes correlated with one another in linear regression plots with high correlation coefficients (>0.97), slopes that are close to 1, and intercepts close to 0 (Table 2). These linear regression plots include the diabetic value of approx 400 mg/dL, which, despite that fact that it lies outside the 75-120 mg/dL glucose value “spread” among all of the samples, is seen in Figures 2 to lie on the best fit regression line.

Possible Reasons for Differences between Studies.

It is not clear why our results differ from those in past studies. One possible reason for the difference is that prior studies did not explore the effects of prolonged pre-analytic periods of tube standing time (4 hours) on glucose levels. It is possible that over this time period, glucose values decrease to a common level. If this explanation holds true, then serum separator and fluoride have no inhibitory effect on glucose metabolism by red cells at (and possibly beyond) this time period. This conclusion conflicts with the prior finding that fluoride exerts its maximal effect over a four-hour period [5,6].

Another possible explanation is the difference in sample size and health status. Prior studies have involved significantly more patients [4,5] with varying medical conditions while our study involved only eighteen volunteers, all of whom are healthy—although one is diabetic. Also, the glucose ranges between the studies are different. Prior studies involved large glucose ranges while our study involved a significant range of glucose values (45 mg/dL, if the diabetic value is excluded and 75-400 mg/dL if this sample is included, giving a range of 325 mg/
dL). Other possible considerations are the effects of medication use and white cell counts, both of which can affect glucose levels and were not considered in this study. We note that our study included individuals of a variety of different racial backgrounds which occurred in the prior studies as well. There was a preponderance of females in the study (13 of the total 18), but it is not clear how this would affect the similarity of glucose levels in all three tube types. Further investigation of the reasons for the difference in findings is warranted.

**Stability of Glucose Values in the Three Different Tubes.**

**Reproducibility of Glucose Values in the Blood Tubes after Initial Analysis.** We also investigated whether glucose values would change when the blood tubes were refrigerated over a prolonged period of time, specifically for five days in this study. Because in each blood tube, with the possible exception of the red-top tube with serum separator, there is some contact between red cells and serum, despite prior centrifugation, that can decrease the glucose levels. This can affect the obtained values if repeat analysis is requested. While past studies have been performed on aliquoted blood samples that are incubated at room temperature for different times, there appears to be no study that examines the reproducibility of glucose values in analyzed samples that are then stored refrigerated over prolonged periods of time.

This objective of our present study is also part of a more general study wherein we are investigating the stability of values determined for commonly ordered, critical serum analytes upon initial analysis and then after the samples are stored at 4°C for periods of ≥ 7 days. These include electrolytes (sodium, potassium, chloride, calcium) [7], BUN, and creatinine [8]. We have found thus far that the serum values over wide ranges for each of these analytes are reproducible, as measured by their coefficients of variation (CVs).

In this study, we find that the lowest CVs for repeated determinations are for red-top tubes with serum separator, and the next-lowest are for grey-top tubes. By a wide margin, the highest CVs are found in the green-top tubes because of the steady decrease in glucose concentrations, presumably due to metabolism of serum glucose by red cells. Throughout the entire five day period of the study, a linear regression analysis showed there was an excellent correlation between glucose levels in red-top and grey-top tubes as exemplified in Table 3 by the high correlation coefficients, the slopes of close to 1, and the Y-intercepts close to 0 (see grey-red entries in Table 3 for days 1 and 5). This finding indicates that red-top or grey-top tubes can be used reliably for the re-assay of glucose concentrations. Because the mean CV for the red-top tubes is lower than for the grey-top tubes, red-top tubes may be slightly more preferable for re-assay although the difference between their mean CVs of 1 and 3 percent, respectively, is small, and the CV values are likewise low for both tube types.

We also note that the high CVs for the green-top tubes is due to the steady decrease in the glucose concentration as revealed by the linear regression analysis that shows that glucose values in these tubes correlate well with those of red- and grey-top tubes, i.e., from Table 3, high correlation coefficients, and slopes close to 1. Only the Y-intercepts have large values (e.g., 32). It is this consideration that rules out use of green-top tubes for serum glucose re-assay.

**References**