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RESEARCH ARTICLE

Preanalytical Variables Influence the Accuracy of Glucose Testing for Diagnostic Purposes

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ABSTRACT

Background: The accuracy of test outcomes is essential for the quality of patient care. To demonstrate the effect of processing delays on glucose levels, we evaluate the stability and decline of glucose in whole blood samples obtained in EDTA vacutainers, which are stored at varying temperatures, as well as the rate of glucose degradation in different color-coded vacutainers.

Methods: Whole blood collected via venipuncture into the vacutainer was carefully mixed to ensure homogeneity. In the temperature study, glucose concentrations were measured at the outset and subsequently at 30-minute intervals in samples preserved at different temperatures. For the study involving color-coded vacutainers, glucose levels in the specimens were assessed initially and at various intervals.

Results: In general, the concentration of glucose in stored whole blood decreased over time. The decline was most significant in the specimen kept at 37 °C when compared to those stored at 24 °C and at ice (0 °C). In the color-coded analysis, all vacutainers, with the exception of the gray top, exhibited a statistically significant decrease in glucose levels. The gray top vacutainer retained as much as 94.7±2.0% of its original glucose concentration even after a duration of 24 hours.

Conclusion: The gray top vacutainer preserves glucose levels to ensure diagnostic precision in situations where a delay in specimen processing is anticipated.

Keywords: Preanalytical variables, Vacutainer, Glucose.

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INTRODUCTION

Preanalytical variables, which are defined as the time interval between the ordering of a test and the analysis of the specimen, account for 32-75% of erroneous laboratory results [1,2]. Established guidelines for specimen collection and handling are crucial to ensure the accuracy of test results, which is vital for delivering quality care [1-4]. The consequences of these errors may involve the necessity for repeat specimen collection, incorrect diagnoses or delayed treatments, increased costs, and potentially even mortality.

Blood glucose levels play a critical role in the monitoring and diagnosis of diabetes. The consistency of blood glucose in whole blood samples is essential for the precise diagnosis of diabetes mellitus and for informed clinical decision-making. The American Diabetic Association (ADA) has established criteria for diagnosing diabetes mellitus, which include testing samples for either (i) fasting blood glucose (FBG) ≥126 mg/dL, (ii) the presence of diabetic symptoms such as polydipsia, polyuria, or unexplained weight loss in conjunction with random blood glucose (RBG) ≥200 mg/dL, (iii) a 2-hour post-load oral glucose tolerance test (OGTT) ≥200 mg/dL, or (iv) measuring glycated hemoglobin A1c (HbA1c) ≥6.5% [5-7]. The HbA1c level indicates the patient's average blood glucose levels over the past three to four months. Unlike FBG and RBG, HbA1c is more stable. Measurements of FBG and RBG are more prone to preanalytical variability, influenced by factors such as stress, physical activity, diet, and the use of color-coded vacutainer tubes for sample collection. It is important to understand that 'random' refers to measuring blood glucose at any time of the day without consideration of the last meal, while 'fasting' indicates testing for blood glucose at least 8 hours after the last meal. Additionally, it is important to note that the diagnosis of diabetes necessitates two abnormal results from two different tests (glucose and HbA1c) conducted on the same day or two abnormal results from samples taken on

different days, provided there is no clear evidence of hyperglycemia [6].

Consequently, it is crucial to uphold rigorous protocols for the collection, storage, and handling of specimens prior to analysis to guarantee the precision of results. The preanalytical factors can differ markedly, encompassing the type of vacutainer utilized, methods of sample collection, handling procedures, transportation conditions, and temperature during storage. These factors can affect test outcomes, potentially resulting in erroneous diagnoses, inadequate clinical decision-making, ineffective treatments, and diminished quality of care.

Adhering to established protocols and guidelines, such as those issued by organizations like the Clinical and Laboratory Standards Institute (CLSI) [4], is vital for preserving the integrity and accuracy of diagnostic tests and safeguarding the quality of care provided to patients. The repercussions associated with laboratory errors stemming from insufficient compliance with established protocols can be considerable, including the necessity for repeat specimen collections, misdiagnoses, delays in treatment, increased healthcare expenditures, and potentially loss of life.

In this study, we investigated the stability of glucose concentrations over time in whole blood samples collected in EDTA tubes that were either stored or incubated at various temperatures, as well as in whole blood contained in four distinct color-coded vacutainers maintained at room temperature. The aim was to ascertain the timeframe within which these specimens should be analyzed to ensure accurate blood glucose readings and to identify which types of vacutainers exhibit resistance to fluctuations in glucose levels over time. Understanding the depletion trends of color-coded vacutainers is critical for ensuring reliable measurements and enhancing our comprehension of the influence of preanalytical variables on laboratory errors in the context of glucose testing.

MATERIALS AND METHODS

The research received approval from the Institutional Review Board (IRB) of the University, reference number 22/03-0031. Whole blood samples were collected from healthy donors via venipuncture and were gently mixed by inverting them eight times back and forth prior to analysis. Glucose concentrations were initially evaluated using a professional monitoring glucose blood meter, with further assessments conducted at 30-minute intervals from aliquoted samples stored at temperatures of 4°C, approximately 24°C (room temperature), and 37°C. Changes in glucose levels over time were documented. This process was repeated, and glucose was measured as previously outlined for a period extending up to 4 hours. The data from each measurement was normalized against the initial value, with the mean and error calculated and represented graphically. All glucose measurements were performed using a McKesson TRUE METRIX PRO Professional Monitoring Blood Glucose Meter. As stated by the manufacturer, this meter is capable of measuring blood glucose levels within a confidence interval ranging from 20 to 600 mg/dL.

In the case of the color-coded vacutainers, whole blood samples were again collected via venipuncture from healthy donors into blue, green, purple, and gray-top vacutainers. The samples were gently mixed as previously described to ensure consistency prior to glucose measurement. Glucose levels were evaluated immediately (at time zero) and subsequently at 1-hour intervals for a total duration of 4 hours, with an additional measurement taken after 24 hours from collection at room temperature (~24°C). At each measurement interval, 0.5 ml of whole blood from the gray top vacutainer was micro centrifuged at 6000 rpm for 2 mins to separate the plasma from the cellular components. The plasma obtained from the gray top was used for glucose measurements. Glucose levels were assessed using a standard glucometer, and all data were normalized to the initial value (100%) for direct comparison. Statistical analyses, including averages, were computed, and error bars were included to indicate variability. The initial measurement at time zero served as the baseline, and subsequent measurements for each specimen were normalized against this baseline. The mean percentage change at various time intervals was statistically analyzed using the Wilcoxon signed-rank test, with a P value greater than 0.05 for a two-tailed analysis employed to assess the significance of the changes, with values exceeding 0.05 deemed not significant.

RESULTS

Glucose depletion was observed at different temperatures. At 37°C, a more pronounced reduction was recorded, with an approximate decrease of 13.00% per hour, compared to 8.00% at 25°C and 2.00% at 4°C. This indicates that the metabolic processes of the cells are affected by temperature, leading to a faster rate of glucose consumption at higher temperatures, as demonstrated in figure 1.

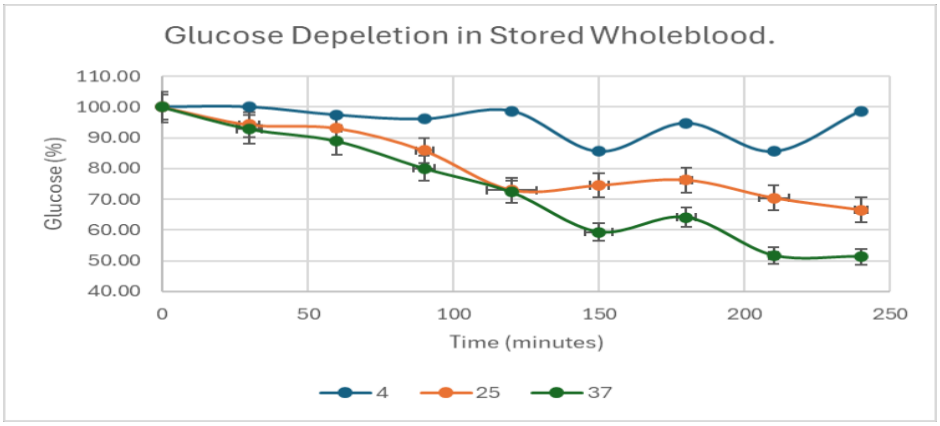

Figure 1. The graph depicting glucose depletion illustrates the average glucose levels across various temperatures.

In figure 3, all vacutainers exhibited a significant decrease in glucose levels over time, showing considerable variability among the various tube types. The Gray-top vacutainer demonstrated the highest stability, retaining 94.7±2.0% of its initial glucose concentration after 24 hours. In contrast, the blue, green, and purple vacutainers experienced a marked reduction in glucose, especially after the first hour, with glucose levels declining to 36.5±4.0%, 22.6±3.8%, and 22.4±3.3%, respectively, during the same period. Within the first 5 hours, the rate of glucose decline was most pronounced in the blue and green vacutainers, with significant reductions observed by the third hour. In figure 2, whole blood collected and stored at room temperature in the four distinct color-coded top vacutainers showed a measurable decrease in blood glucose over time, except for the gray-top vacutainers. Glucose depletion was significant (p-value < 0.05) in the blue, green, and purple top vacutainers after 1 hour and became undetectable, falling below the 20 mg/dL limit of detection for the assay at the 24-hour mark. Generally, these tubes serve as vital instruments in clinical laboratories for the collection and analysis of blood. Each tube's distinct color signifies the specific type of anticoagulant it contains and its designated purpose.

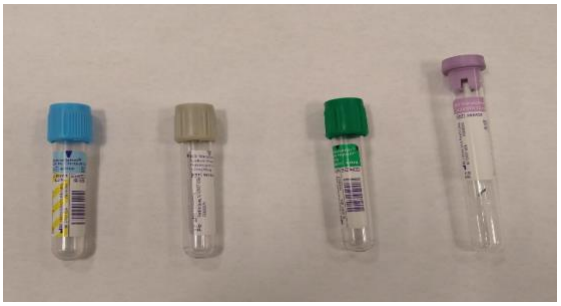

Figure 2 : The color-coded Vacutainer tubes (left to right), blue, gray, green & purple tops utilized in the study.

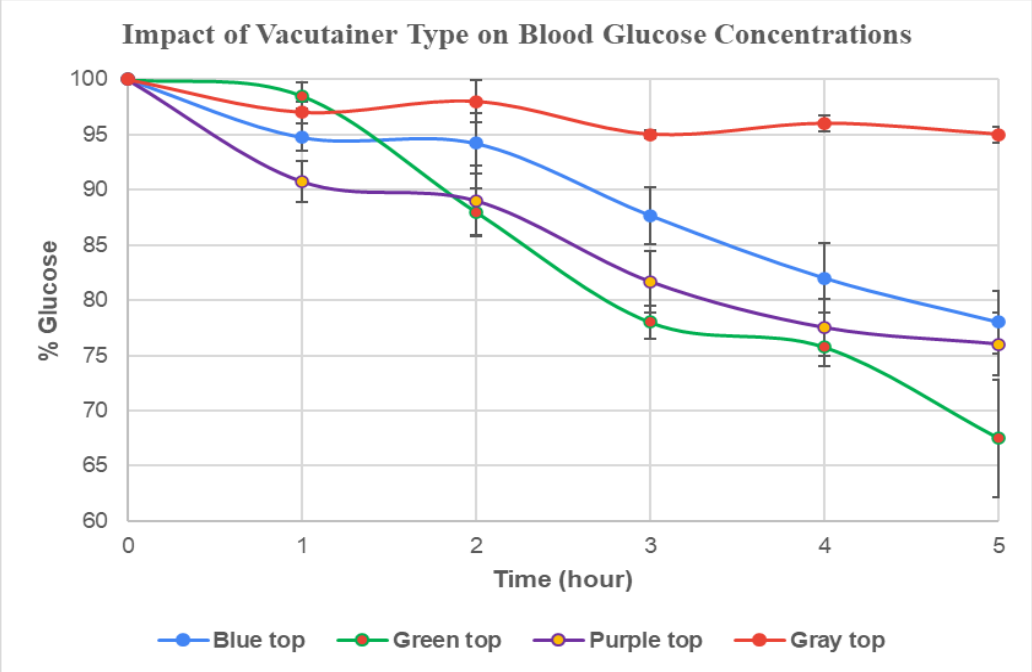

Figure 3: Glucose concentrations (mean calculated from four repetitions conducted across four distinct days) in whole blood samples collected using four different colored top vacutainers.

DISCUSSION & CONCLUSION

Glucose measurements taken from gray top vacutainers showed consistency both after five hours and up to 24 hours post-draw. In contrast, the blue, green, and purple tops displayed notable fluctuations in glucose levels when there was a delay in measurement. Specifically, after one hour, glucose concentrations dropped by as much as 10%, and by five hours, the decrease reached 40%. This reduction corresponds with the 7% decline documented in existing literature at similar time intervals [7, 8]. In clinical laboratory settings, blood glucose measurement can be performed using serum, plasma, or whole blood. To ensure the preservation of blood glucose levels without significant loss, it is essential to promptly separate plasma or serum from the packed cells and store them under refrigeration [9]. Any delay in glucose assay for whole blood may result in inaccurate measurements due to cellular depletion.

One potential limitation is the variability that stems from the detection threshold. When measurements are below the 20 mg/dL limit, the meter displays "Lo" for glucose blood tests. As a result, 20 mg/dL was included in the average calculation in these cases. Nevertheless, the results indicate that whole blood collected in gray vacutainers is the optimal choice for glucose measurement when delays in processing are anticipated. The gray top contains sodium fluoride, which serves as both an anticoagulant and a glycolysis inhibitor, thereby preserving approximately 95% of glucose concentrations in whole blood for up to 24 hours.

This research reinforces the established understanding that the gray top vacutainer is the most effective for maintaining glucose integrity and stability, making it the preferred option when there is a delay in specimen processing [9-11]. As illustrated in figure 1, the green, blue, and purple top vacutainers can also be used for glucose analysis, as long as the whole blood sample is

processed within one hour of collection. If there is a delay, particularly one that exceeds one hour before measurement, the glucose results may be inaccurately low. The whole blood in the green, blue, and purple top vacutainers must be centrifuged to separate the plasma from the packed cells to preserve glucose concentration in the plasma if a gray top is not available. Nakanga et al. [9] recommended using an EDTA tube, provided the specimen is placed on ice immediately and analyzed or centrifuged within six hours. Furthermore, any vacutainer can be used if plasma or serum for glucose measurement at room temperature is quickly separated from the red cells. In stored whole blood, glucose levels decrease by about 7% each hour. Generally, plasma glucose concentration is approximately 10-15% higher than that of whole blood due to the ongoing metabolic activities of the cells [6, 7].

The color-coded tops indicate the different anticoagulants found in each vacutainer. Typically, the purple top vacutainer, which contains EDTA as its anticoagulant, is primarily used in the hematology department of clinical laboratories for analyzing specimens for complete blood count (CBC), erythrocyte sedimentation rate (ESR), and other related tests. Furthermore, it is also utilized for specific chemistry tests, such as hemoglobin A1c (HA1c), procalcitonin, and the evaluation of immunosuppressant drug levels (including tacrolimus and cyclosporine) in transplant patients. The green top vacutainer, which is treated with heparin anticoagulant, is appropriate for standard chemistry tests.

The blue top vacutainer, which contains sodium citrate, is employed for the assessment of coagulation markers such as Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT), D-dimer, von Willebrand factor, and C and S proteins, among others.

The gray top vacutainer, which incorporates sodium fluoride along with sodium or potassium citrate, is utilized for evaluating glucose and lactate levels [12]. Sodium fluoride and potassium oxalate are crucial in preserving the integrity of blood samples. Potassium oxalate interacts with calcium, inhibiting its participation in the coagulation cascade, thus acting as an anticoagulant. Fluoride inhibits enolase, a key enzyme in the glycolytic pathway that aids in the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Gambino R. [11] observed that fluoride by itself is not a potent glycolysis inhibitor. Nevertheless, when used in conjunction with oxalate, fluoride effectively maintains plasma blood glucose levels. In cases where a gray tube is unavailable, the American Diabetes Association (ADA) advises that plasma should be chilled and separated from cells within 30 minutes to preserve glucose levels.

This study underscores the importance of proper specimen collection, handling, and prompt processing to obtain accurate results, which are vital for screening, diagnosis, quality of care, and overall medical expenses. In scenarios where immediate glucose measurement is not possible, diagnostic accuracy is upheld when the glucose concentration in whole blood is preserved in a gray top vacutainer or processed immediately through centrifugation to separate plasma from cellular components.

ABBREVIATIONS

EDTA	Ethylenediaminetetraacetic acid
HbA1c	Glycated hemoglobin A1c
OGTT	Oral glucose tolerance test
ADA	American Diabetic Association
CLSI	Clinical and Laboratory Standards Institute

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AUTHORS' CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this case.

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