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Short Communication

The impact of different sample matrices in delayed measurement of glucose

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ABSTRACT

Objectives: This study was designed to compare glucose values in serum or lithium-heparin samples immediately centrifuged with those paired specimens collected in tubes containing Naf-KOx and centrifuged and analyzed 2.5 h after collection.

Methods: Three blood samples were drawn from 20 volunteers. Blood samples collected in tubes with and clot activator and gel separator but without anticoagulant (SST) as well as those collected in tubes containing Lithium-Heparin and gel separator were centrifuged within 30 min and analyzed 2 h thereafter. Blood samples drawn in tubes containing the glycolysis inhibitor NaF-KOx were centrifuged after 2.5 h and then analyzed.

Results: The glucose median value was 4.72 mmol/L in SST tubes, 4.67 mmol/L in lithium-heparin and 4.44 mmol/L in NaF-KOx tubes. The difference between SST and lithium-heparin tubes was not statistically or clinically significant, whereas that between SST and Naf-KOx tubes was both analytically and clinically meaningful, exceeding the current quality specifications for glucose measurement.

Conclusions: The rapid centrifugation of blood collected in serum or lithium-heparin tubes with gel separator is seemingly more reliable for delayed measurement of glucose compared to the use of blood tubes containing NaF-KOx.

1. Introduction

Glucose measurement is a mainstay for the diagnosis of diabetes mellitus, impaired fasting glucose, impaired glucose tolerance and for the screening and diagnosis of gestational diabetes mellitus [1–3]. Therefore, the measurement of this analyte must be both accurate and precise for appropriate patient classification according to the well-established international guidelines.

Laboratory professionals and in vitro diagnostic (IVD) companies have placed large efforts for improving the routine measurement of plasma or serum glucose. Due to the use of enzymatic methods and sophisticated analyzers with stable optics, electronics and other components, clinical laboratories have achieved an impressively low intra-laboratory imprecision (CV), frequently lower than 1%. Unlike the analytical phase, many pre-analytical issues related to glucose testing have not been completely resolved so far [4,5].

A well-known challenge in the accurate measurement of glucose is the gradual decay of its concentration in blood specimens, which is mostly attributable to erythrocyte metabolism after collection, especially during transportation and processing [6]. Since red blood cells (RBCs) metabolize glucose through the glycolysis pathway, glucose concentration in whole blood decreases approximately by 0.39 mmol/L per hour [7]. The pre-analytical reduction of glucose in laboratory specimens during the first period (i.e., 1–2 h) after collection is hence regarded as a very frequent source of bias in diagnostic testing which, in the worst possible clinical scenario, may ultimately led to underdiagnosing diabetes.

Consolidated evidence has been brought that the large majority of laboratory errors are due to extra-analytical issues, which are especially attributable to inaccurate or inappropriate activities during collecting, management and transportation of biological specimens [8].

According to innovative paradigms of laboratory organization, progressing towards an increasing popularity of "hub-and-spoke" facilities, delayed analysis of blood samples is becoming commonplace in routine practice, wherein whole blood samples are collected in peripheral collection centers or "spoke" laboratories and then transported to large diagnostic centers in which a large number of tests and specialized analyses have been consolidated. Depending on the condition of sample transportation (i.e., time, temperature, humidity), it cannot be excluded that the quality of serum or plasma may be impaired and the following test results may be biased [9].

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Table 1
Effects of delayed (i.e., 2.5 h) centrifugation of blood samples collected in blood tubes containing sodium fluoride combined with potassium oxalate (NaF-Kox) for glucose measurement (mmol/L).

	Tubes	Min	1st Quartile	Median	95% CI	3rd Quartile	Max	IQR	P
Glucose	SST	4.05	4.62	4.91	4.72 to 5.44	5.50	6.55	0.88	–
Glucose	Li-heparin	3.99	4.62	4.68	4.72 to 5.38	5.54	6.55	0.93	1.000
Glucose	**NaF-Kox**	3.77	4.29	4.63	4.38 to 4.88	5.11	6.16	0.82	<**0.0001***

Min: lowest value; CI: confidence interval; Max: highest value; IQR: Interquartile Range.
Statistically significant differences in bold.
* Statistically significant variation (p value < 0.05) compared to glucose STT.

Two essential approaches have been historically suggested to prevent or limit the glycolytic consumption of glucose in whole blood sample, entailing the rapid physical separation by centrifugation of serum or plasma from metabolically active cellular fractions (especially RBCs and leukocytes) and the addition of various inhibitors of glycolysis (e.g., fluoride) to the specimen [10].

Therefore, the aim of this study was to analyze the pre-analytical stability of glucose by comparing results of glucose in samples collected without glycolysis inhibitors, immediately centrifuged and analyzed after 2 h or in samples collected in tubes containing a glycolysis inhibitor (sodium fluoride combined with potassium oxalate; NaF-KOx), centrifuged and analyzed 2.5 h after collection.

2. Materials and methods

The study population consisted of 20 ostensibly healthy volunteers recruited from the laboratory staff (8 males and 12 females; mean age 40 ± 10 years). Blood was always drawn by a single experienced nurse in three different collection tubes, i.e., one tube with gel separator but without anticoagulant and clot activator (SST) (Venosafe®, Terumo Europe N·V, Leuven Belgium), one tube containing Lithium-Heparin and gel separator (Li-Heparin) (Venosafe®, Terumo Europe N·V, Leuven Belgium), and one tube containing KOx as anticoagulant and NaF as glucose stabilizer (VACUETTE® Glycolytic Inhibitor Tubes, Greiner Bio-One).

The tubes containing clot activator and gel separator but without anticoagulant (SST), as well as those containing Lithium-Heparin (Li-Heparin) and gel separator were centrifuged immediately (i.e., within 30 min from collection) and then maintained at room temperature (RT) for 2 h, after which glucose measurement was performed. Blood samples containing the additive NaF-KOx were instead maintained at room temperature (RT) for 2.5 h, after which they were centrifuged and glucose was immediately (i.e. within 5 min) measured.

All samples were tested using the automated clinical chemistry analyzer ARCHITECT PLUS (Abbott Diagnostics, Roma, Italy) according to manufacturer's recommendations. Blood glucose was hence measured with the reference hexokinase method. The analytical imprecision of this assay (expressed as coefficient of variation, CV) is <1%, as declared by the manufacturer and further confirmed by a comprehensive analytical evaluation of the analyzer [11]. All samples were tested on the same analyzer, using an identical lot of reagents in order to prevent lot-to-lot variability. The significance of differences between paired samples (i.e., serum; lithium heparin and NaF-KOx) of the same volunteer was evaluated with Wilcoxon signed-rank test, using Analyse-it (Analyse.it Software Ltd., Leeds, UK). Results were considered significant when the p value was <0.05. When the difference of values measured in the tubes (SST) and in the other tubes (i.e., Li-Heparin and NaF-KOx) was found to be significant, the relative percentage variation was analyzed by means of Bland-Altman plots and then compared with the current quality specifications for desirable bias derived from the intra-individual and inter-individual variations [12].

The results were finally reported as lower value, 1st Quartile, median and 95% Confidence Interval (95% CI), 3rd Quartile, upper value, interquartile range (IQR). All samples were anonymized before analysis. All subjects give an informed consent for being enrolled in this investigation. The entire study was performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

3. Results

The main results of this study are shown in Table 1. The median value of glucose collected in SST tubes (85 mg/dL; 4.72 mmol/L) was

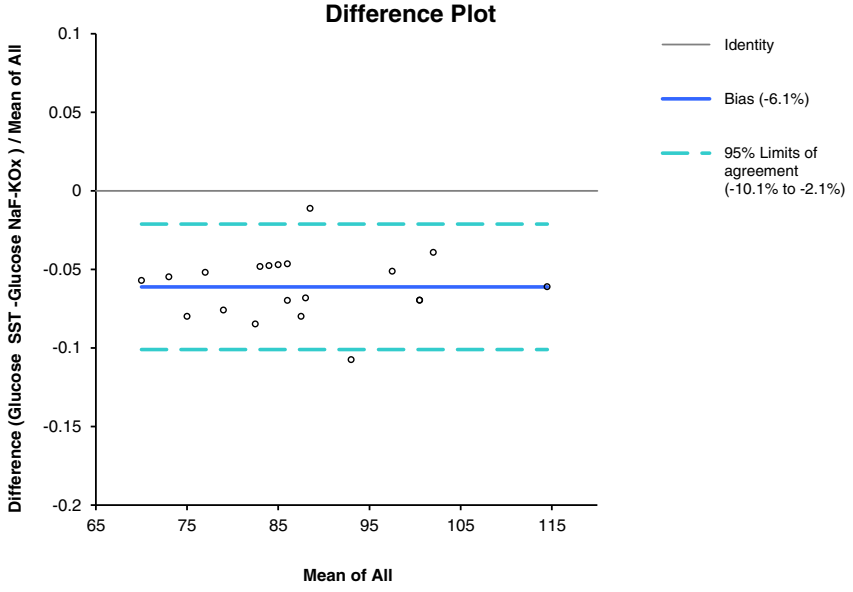

Fig. 1. Mean % bias and 95% limits of agreement for glucose measured in SST tubes versus glucose measured in NaF-KOx tubes.

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