Contents lists available at ScienceDirect

Primary Care Diabetes

journal homepage: http://www.elsevier.com/locate/pcd

Original research

Which sample tube should be used for routine glucose determination?

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
2 November 2015
Accepted 5 November 2015
Available online 4 December 2015

Keywords:
Glucose
Hexokinase
Glycolysis inhibition
Sodium fluoride
Citrate acidification
Preanalytical conditions
Stability

ABSTRACT

Background: Glucose is one of the most frequently requested analytes in clinical laboratory. Blood glucose analysis is affected from in vitro glycolysis. In order to determine the most suitable blood collection tube for this purpose we have compared different tubes: sodium fluoride, lithium heparin, sodium fluoride/citrate buffer containing tubes and serum with clot activator tube for the measurement of glucose when the tube has been kept at room temperature (RT) for up to 4 h.

Methods: Venous blood was collected from 49 healthy volunteers into Sarstedt S-Monovettes for glucose analysis. Reference plasma glucose was determined in a lithium heparin tube and immediately placed in an ice/water slurry. Within 10 min it was centrifuged at 4 °C and plasma was separated from the blood cells. Samples have been preserved at RT for 1, 2 and 4 h after drawing. Glucose has been determined using a hexokinase method.

Results: Glucose levels tested in a serum with clot activator tube, in lithium heparin and in sodium fluoride/sodium EDTA tubes when compared with lithium-heparin reference plasma did not meet the desirable bias for glucose (±1.8%) when kept at RT for up to 4 h. GlucoEXACT tubes, when corrected by the Sarsted recommended factor of 1.16, showed a mean (95% CI) bias of +0.96% (0.45–1.47) at 1 h, +1.40% (0.88–1.93) at 2 h and +0.95% (0.44–1.46) at 4 h, reaching the analytical goal for the desirable bias.

Conclusions: Samples collected into GlucoEXACT tubes containing sodium fluoride/citrate buffer liquid mixture are equivalent to those collected in reference plasma tubes avoiding glycolysis completely and within a 4 h delay in plasma separation.

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http://dx.doi.org/10.1016/j.pcd.2015.11.003
1751-9918/

1. Introduction

Accurate measurement of plasma glucose is fundamental for the diagnosis of diabetes an impaired fasting glucose, in particular for gestational diabetes for which HbA1c can't be used for diagnosis [1,2].

The decrease of glucose in blood samples prior to analysis due to ex-vivo glycolysis [3] is the main cause of interference for plasma glucose determination, particularly when the analysis is delayed and when the sample is not kept cold enough and it's not centrifuged immediately after venipuncture [4].

Elimination of in vitro glycolysis is fundamental to ensure accurate results and to reliably diagnose diabetes.

In order to avoid in vitro glycolysis, several inhibitors have been used as an additive in the blood collection tube: sodium fluoride (NaF), sodium or lithium iodoacetate [5], D-mannose [6] and blood acidification [7].

NaF is commonly used but is not a very effective agent on its own as it inhibits the enzyme enolase, which catalyzes a down-stream step of glycolysis. Other up-stream step enzymes remain active which delays glycolysis inhibition for about 4 h until all substrates for up-stream enzymes are depleted [8,9].

In contrast, the acidification of whole blood with citrate buffer seems to immediately inhibit the up-stream enzymes of glycolytic cascade (hexokinase and phosphofruttokinase) [10].

The 2011 guidelines of the National Academy of Clinical Biochemistry (NACB) for blood collection and storage for laboratory analysis for the diagnosis and management of diabetes recommend minimizing the influence of glycolysis on plasma glucose determination by immediately placing the sample tubes in an ice–water slurry and separating plasma from cells within 30 min from blood drawing [11].

As the optimal method of cooling and cold processing blood samples within 30 min is not feasible in routine analysis, the use of tubes containing inhibitors such as NaF with citrate buffer, is recommended in order to prevent early glycolysis [11].

In order to assess which sample is best for blood glucose determination we compared the stability of glucose in serum and plasma samples (lithium-heparin, EDTA with or without glycolysis inhibitor NaF, citrate buffer), comparing them to reference glucose according to ADA–NACB guidelines [11].

2. Materials and methods

49 (16 males, 33 females, mean age 30.3 years, range: 20–59 years) fasting and non-fasting volunteers were recruited.

This study has been performed in accordance with the ethical standards of the revised Declaration of Helsinki and informed consent from volunteers was obtained.

A venous blood sample was obtained from antecubital fossa of the arm using a 21 G (gauge) winged needle set (Nipro SafeToouchTM PSV, ref. PSV SF 21 ET), connected with multiadapter for S-Monovette® (Sarstedt, ref. 14.1205).

The blood has been collected in five different tubes: a S-Monovette serum with clot activator 4.9 mL draw (Sarstedt ref. 04.1934), two S-Monovette lithium heparin 4.9 mL draw (Sarstedt ref. 04.1936), a S-Monovette NaF/Na_2EDTA 2.7 mL draw (Sarstedt ref. 04.1918) and the new S-Monovette GlucoEXACT 3.1 mL draw (Sarstedt ref. 05.1074 001).

All tubes were fully filled; the order of draw according to CLSI GP41-A6 guidelines [12] was serum tube, lithium-heparin and finally tubes containing glycolysis inhibitors.

For S-Monovette GlucoEXACT containing a liquid mixture of sodium fluoride and citrate, it is vital to collect a correct draw volume considering the dilution factor (1.16, reported on tube label).

Gold standard sample: one lithium heparin tube immediately placed in an ice/water slurry, centrifuged within 10 min after withdrawal at 2500 g at 4 °C for 15 min and plasma separated from blood cells in 1.5 mL polypropylene micro tube (Sarstedt, ref. 72.690.001) and kept at 2–6 °C until analysis.

The blood collected in the other tubes was immediately aliquoted after drawing in three 1.5 micro tubes and kept at room temperature (20–24 °C) for 1, 2 and 4 h, respectively. Next they were centrifuged at 2500 × *g* at 4 °C for 15 min, serum and plasma were aliquoted in 1.5 mL polypropylene micro tubes, finally kept at 2–6 °C until analysis.

All samples were analyzed in triplicate using a hexokinase method for measurement of serum and plasma glucose (intra-assay coefficient of variation <1.0%) on a Dimension Vista 1500 system (Siemens Healthcare Diagnostics).

Measurement of Serum-Index, Hemolysis, Icterus and Lipemia (HIL) (ranging from 1 to 8 on Siemens Dimension Vista) was undertaken using a photometric method.

3. Statistical analysis

The data were analyzed for distribution using the Kolmogorov Smirnov test. Parametric and nonparametric methods were used as appropriate. Paired *t*-test or Wilcoxon tests were used to compare glucose levels found in the evaluated tubes in comparison to those found in the gold standard sample, in addition to testing the significance of glucose loss over time. Comparisons of the means of triplicate glucose determinations were made by Passing and Bablok regression. Statistical analyses were undertaken using MedCalc statistical software, version 13.12 (Ostend, Belgium).

4. Results

Glucose determinations were made in triplicate and expressed as mean value for each sample tube and for each time after blood draw.

Median reference glucose from samples collected in lithium-heparin tube, immersed immediately after collection in an ice/water slurry, centrifuged within 10 min from drawing at 4 °C at 2500 × *g* for 15 min and plasma separated immediately after centrifugation in 1.5 mL micro tube, and stored at 4 °C was 4.98 mmol/L (95% CI 4.89–5.10).

Table 1 is a summary of median glucose values and their 95% CI from blood in sample tubes stored at room temperature (RT) (20–24 °C) for 1, 2 and 4 h after drawing and then