Preanalytical sample handling of venous blood: how to ensure your glucose measurement is accurate and reliable

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Abstract
Measurement of blood glucose is a standard biochemical test requiring optimum preanalytical sample handling. Glucose measured in plasma from tubes containing sodium fluoride is recommended but serum from serum-gel tubes may be used in research situations. To help inform best practice, we assessed glucose stability in plasma and serum samples subjected to different preanalytical conditions.

Fasting samples were taken from 10 non-diabetic volunteers into fluoride/EDTA and serum-gel tubes. Whole blood samples were pipetted into aliquots, placed on crushed ice or left at room temperature. Aliquots were centrifuged at 0, 2, 12, 24, and 48 hours.

When neither ice nor centrifuge were available, plasma glucose was stable for 48 hours (96% of baseline); serum glucose degraded to 8% of baseline. When centrifuged and left at room temperature, plasma glucose was stable for 48 hours (101% of baseline) but, by 24 hours, serum glucose had fallen (94% of baseline). The result of un-centrifuged plasma on ice was stable (96% of baseline) at 48 hours; serum glucose had dropped to 92% of baseline by 12 hours. Plasma glucose and serum glucose were constant for 48 hours when separated and placed on ice within 2 hours: plasma glucose 101% of baseline; serum glucose 100% of baseline. Separated serum resting on the serum plug fell to 94% of baseline by 12 hours at room temperature and to 92% of baseline by 48 hours on ice.

Clinically, we recommend that glucose is measured on plasma taken from fluoride tubes. Analysis should be undertaken as soon as practicable. In the research setting, glucose can be measured on plasma or serum but samples must be centrifuged and chilled soon after venepuncture and analysed within 48 hours. Copyright © 2013 John Wiley & Sons.

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Key words
glucose; preanalytical handling; plasma; serum; fluoride; serum gel; reliability

Introduction
Accurate glucose measurements are essential to ensure correct diagnosis and treatment choices in patients with diabetes. Biochemistry laboratories achieve exact results with low within-laboratory imprecision (coefficient of variation [CV]) of 1–2%.¹ ² but correct blood collection tubes and optimum preanalytical sample handling are also essential to ensure accuracy.¹ ³

For clinical and diagnostic purposes, plasma glucose from tubes containing sodium fluoride (NaF) is recommended as it decreases ex vivo glycolysis.¹ ² In the research setting, using serum-gel tubes is often acceptable enabling many biochemical measures to be determined using a single sample.⁴

On a day-to-day basis, there are factors influencing preanalytical sample handling that may negatively impact on accuracy. In the clinical setting, a glucose sample may be taken on serum gel, or the correct tube may be used but the sample taken too late to be couriered to the laboratory and consequently left overnight on the workbench or refrigerated. In the research setting, studies may take place many miles away from the laboratory, delaying time to analysis.

With these scenarios in mind and taking into account whether or not a centrifuge and/or chilling medium were available, we aimed to determine optimal preanalytical sample handling procedures by (1) determining whether plasma or serum is more stable, and (2) assessing the impact of time to centrifugation and storage temperature on the stability of glucose.

Materials and methods
Fasting (overnight) venous blood was taken from 10 healthy non-diabetic adults (glucose range
3.3–6.8 mmol/L) in tubes containing sodium fluoride/ethylene diamine tetraacetic acid (NaF/EDTA) and serum gel (Sarstedt S-Monovette® tubes [Sarstedt, Leicester, UK]).

Baseline measures were taken from both types of blood tube. After venepuncture, one NaF/EDTA and one serum-gel tube was centrifuged and the resulting plasma and serum were taken to the laboratory for glucose measurement.

Remaining samples were split into aliquots and centrifuged at either 0, 2, 12 or 48 hours and were kept at room temperature or placed on crushed ice (see Figure 1 and supplementary methods). Samples were stored at -20°C and analysed as a batch at the Clinical Chemistry Department, Royal Devon and Exeter NHS Foundation Trust (coefficient of variation for glucose measurement 2%), on the Roche P800 analyser using the glucose oxidase/peroxidise method (GOD-POD, Roche Diagnostics, Mannheim, Germany).

We provide results of plasma and serum samples exposed to four different situations: (1) un-centrifuged and left at room temperature; (2) centrifuged and left at room temperature; (3) un-centrifuged and placed on ice; and (4) centrifuged and placed on ice. Figure 2 shows the stability over time for each of the scenarios.

### Statistical analysis

Results are presented as mean percentage change from baseline (sample centrifuged, separated and frozen at time-point zero) ± 95% confidence interval (CI). Differences between the baseline and the level of glucose at time-points were assessed by the Wilcoxon signed-rank test.

### Results

Baseline glucose levels in plasma and serum were similar: mean 4.5 mmol/L vs 4.6 mmol/L respectively.

#### Un-centrifuged sample at room temperature

NaF/EDTA un-centrifuged samples left at room temperature dropped to 96% of baseline (p=0.07) at 48 hours.

#### Un-centrifuged serum-gel samples

Un-centrifuged serum-gel samples left at room temperature had dropped to 90% within 2 hours (p=0.005) and to 8% by 48 hours (p=0.005).

#### Centrifuged plasma and serum at room temperature

When separated, pipetted into aliquots and left at room temperature, NaF-plasma was stable at 101% of baseline at 48 hours (p=0.2). Separated and pipetted serum left at room temperature fell to 94% by 24 hours (p=0.2). By 48 hours there was a drop to 89% (p=0.03).

Serum samples centrifuged but left on the serum plug were constant for just 2 hours when left at room temperature, 98% of baseline (p=0.05), decreasing to 87% at 24 hours (p=0.02).

#### Centrifuged plasma and serum with ice storage

Un-centrifuged NaF-plasma placed on ice decreased to 96% by 48 hours (p=0.1). Un-centrifuged serum gel was constant for 2 hours, 99% of baseline (p=0.3), decreasing to 87% at 24 hours (p=0.005).

#### Un-centrifuged sample with ice storage

Un-centrifuged NaF-plasma placed on ice decreased to 96% by 48 hours (p=0.1). Un-centrifuged serum gel was constant for 2 hours, 99% of baseline (p=0.3), decreasing to 87% at 24 hours (p=0.005).

#### Centrifuged plasma and serum with ice storage

Both NaF-plasma and serum, removed from cells, pipetted into aliquots and placed on ice, were constant for 48 hours (101% [p=0.2] and 100% [p=0.5] of baseline respectively).
Sample handling: ensuring glucose results are accurate and reliable

Serum samples that had been centrifuged but left on the serum plug were stable at all time-points when placed on ice.

**Discussion**

Correct preanalytical sample handling of blood for glucose analysis is essential as the important diagnosis of diabetes relies on accurate glucose reporting. NaF is the recommended preservative of choice for glucose measurement. Yet, as fluoride inhibits glycolysis by the inhibition of enolase in the later part of the glycolytic pathway but not the enzymes upstream of enolase which continue to metabolise glucose 6-phosphate, glycolysis still occurs within the first hour and for up to 4 hours. For this reason, previous studies recommend that samples are stored on ice-water and analysed within 1 hour or are immediately centrifuged.

We did not see a major drop in plasma glucose within the first 2 hours and confirmed that glucose, measured on NaF-plasma, is stable for 48 hours under all conditions studied. NaF-plasma should be used for clinical and diagnostic purposes but storage on ice-water, analysis within 1 hour or immediate sample separation are not essential. Plasma kept overnight on the workbench or in the fridge may be accepted for analysis the following day.

Previous reports suggest measuring glucose on serum is not recommended for diagnostic or treatment purposes as the glucose content may be lower than in plasma samples. Comparable to another study, our findings did not support this.

Immediate sample separation and refrigeration are recommended if serum-gel tubes are used. We confirm that serum is not stable when left un-centrifuged, but is stable for up to 48 hours at room temperature when removed from cells soon after venepuncture. In the research setting where a centrifuge and ice are available, using serum-gel tubes is acceptable but uniform sample-handling must take place.

Possible limitations to our study are the narrow range of glucose levels and analysing frozen samples. Conversely, all samples were analysed at the same time by the same operator and plasma and serum may be stored at -20°C for long periods of time.

**Conclusion**

In the clinical setting where a centrifuge and/or ice are rarely available, we recommend that current guidelines are followed: glucose should be...
measured in plasma taken from tubes containing NaF. Centrifugation and chilling of the sample within the hour are neither practical nor essential but processing of the sample in a timely manner is to be encouraged.

In the research setting where a centrifuge and ice are more readily available, we suggest that either NaF or serum-gel tubes can be used. Plasma and serum need to be centrifuged and chilled immediately but both will then remain stable for 48 hours.

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Declaration of interests
There are no conflicts of interest declared.

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