Published in final edited form as:
Clin Chem. 2009 May ; 55(5): 850–852. doi:10.1373/clinchem.2009.126037.

Stabilization of Glucose in Blood Samples: Why It Matters

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In this issue of *Clinical Chemistry*, Gambino et al. (1) describe careful studies of a blood-collection container that stabilizes the concentration of glucose in blood samples. At a time of an increasing disease burden attributable to diabetes and the use of lower glucose concentrations than in the past for the diagnosis of diabetes, this study and its findings are especially important.

Measurements of glucose are used worldwide to diagnose diabetes and to identify patients at risk of developing diabetes [e.g., (2,3)]. For both diagnosis and risk assessment, fixed cutpoints of plasma glucose concentrations are used to classify patients and to make decisions regarding management. For this reason, all steps in the analytical process require careful attention.

Sources of Error in Measurements of Plasma Glucose

Clinical chemists in hospital laboratories and diagnostic companies have made great strides in improving the measurement of glucose. With the use of enzymatic methods and sophisticated analyzers with stable optics, electronics, fluid handling, and other components, central clinical laboratories routinely achieve an astoundingly low within-laboratory imprecision (CV) of 1%–2%. [Glucose meters do not fare so well and are not recommended for diagnosis of diabetes (4).] By contrast, the preanalytical issues surrounding glucose measurements have not been solved.

The loss of glucose in blood samples has been studied for many years (5). Glucose is lost through glycolysis at a rate of 5%–7%/h at concentrations near the reference interval. In absolute terms, a loss in glucose of about 0.67 mmol/L (12 mg/dL) occurs at a concentration of 5.55 mmol/L (100 mg/dL) after 2 h at room temperature (6). Higher rates of loss occur commonly, such as with increased ambient temperature and in samples with high white blood cell counts. In 1923, Major introduced potassium fluoride as a potent inhibitor of glycolysis

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Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:
Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: W.C. Knowler, Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases.
Expert Testimony: None declared.

(7), and fluoride, usually as NaF, has been used for decades to inhibit glycolysis in blood samples. Less well appreciated is the observation (6) that NaF has little or no effect on the rate of glycolysis during the first 1–2 h or more after blood is collected. Such findings appear to reflect the fact that the major glycolytic enzyme targeted by fluoride, enolase (phosphopyruvate hydratase, E.C. 4.2.2.11), is located distally in the glycolytic pathway. Thus, even in the presence of fluoride, glucose is phosphorylated by available ATP, and the glucose 6-phosphate formed is further metabolized until equilibria are reached in reactions proximal to enolase in the glycolytic pathway (8).

The preanalytical loss of glucose from samples during the first 1–2 h after collection is likely a larger source of error than is the analytical error in clinical laboratories. Although some intermethod differences remain, CVs for individual methods are typically ≤2% among laboratories for survey samples, and the all-method CVs are typically 3% [e.g., (9)]. By contrast, the loss of glucose, with or without fluoride, is 5%–10% or greater at 1–2 h after sample collection (1,7,8).

The handling of blood samples collected for glucose analysis has been little studied in recent years, perhaps reflecting a mistaken belief that use of NaF has solved the preanalytical problems. It has been known for many years that the loss of glucose can be prevented by placing blood-collection tubes immediately into an ice slurry, centrifuging the samples with minimal delay in a refrigerated centrifuge, and removing the plasma promptly. Use of an ice slurry, however, is not a practical solution in modern healthcare.

Now, Gambino et al. (1) have reported that the stability of glucose seen with an ice slurry can be achieved with use of a blood-collection tube that was described in a US patent more than 20 years ago but is little known in most laboratories. The use of such a sample-collection device has great appeal given the current need for accurate measurements of glucose and raises issues that require attention and resolution.

Diagnosis of Diabetes

Cutpoints of plasma glucose concentrations are based on carefully designed studies, performed over many years, that provide the evidence base for the diagnosis of diabetes (2). Thus, a fasting plasma glucose concentration ≥7.0 mmol/L (≥126 mg/dL), rather than the various, higher cutpoints used in the past, is broadly accepted as diagnostic of diabetes (when observed on 2 or more occasions). Glucose concentrations above this new cutpoint predict the later development of pathologic changes, such as diabetic retinopathy. Similarly, for gestational diabetes, the recent Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study (10) has defined the risk of adverse neonatal and maternal events as a function of glucose concentrations. As with studies that led to the lower cutpoint for the diagnosis of diabetes, the HAPO study has shown that the risk of unwanted events begins to increase at lower concentrations than have been used for the diagnosis of gestational diabetes in the past. This finding is leading to a reexamination of the diagnostic criteria for gestational diabetes. For the diagnosis of diabetes and of gestational diabetes, accurate measurements of glucose are increasingly critical, because decisions are being made at the lower concentrations seen in large numbers of people worldwide. Even small errors will lead to the misclassification of many patients.

In view of the large losses of glucose in blood samples and the fact that fixed cutpoints are used for diagnosis of diabetes, it is important to consider how closely sample-handling procedures in routine practice agree with those used in the studies that defined the cutpoints for diabetes diagnosis. If samples are handled differently in routine practice than in the studies that defined diabetes cutpoints, patients will be misclassified in practice. Again, as with analytical error, the number of patients misclassified from this preanalytical error is potentially extremely large now that diagnostic cutpoints are at the lower glucose concentrations found in

a large proportion of the population. Current diagnostic cutpoints will need reevaluation if there are widespread changes in clinical procedures, such as by immediate icing of tubes or by use of the collection tube described by Gambino et al. (1). Such changes would produce higher values for measured glucose than currently obtained by procedures that do not inhibit glycolysis promptly.

In the HAPO study of pregnant women (10), blood samples for measurement of fasting plasma glucose were placed immediately in an ice slurry, thus stopping glucose metabolism promptly and completely (1,8). To obtain glucose concentrations that are comparable to those in the HAPO study and to allow estimation of the patient's risk of adverse pregnancy outcomes from HAPO data, clinical laboratories will need to (*a*) provide very rapid processing of samples, (*b*) use an ice slurry, or (*c*) use a glucose stabilizer that inhibits glucose metabolism promptly. Doing so, however, will produce results higher than are appropriate for use with the diagnostic cutpoints for the diagnosis of diabetes in nonpregnant adults.

Further Implications of a Truly Stabilized Glucose Sample

The instability of glucose in blood, with or without NaF, not only introduces errors in the classification of individual patients but also introduces noise into epidemiologic data. Stabilization will avoid this noise. As with most forms of variability in clinical practice, the variability in the time between blood collection and analysis is worth addressing. This variation in time is, to a large extent, unavoidable outside of highly controlled settings, but stabilization of the glucose in the sample can remove the effect of this variability.

The variable loss of glucose in samples may have led us to overestimate the within-person biological variability of glucose. This possibility warrants reexamination. Similarly, the reported irreproducibility of the oral glucose tolerance test also warrants reexamination with the use of blood-collection procedures or tubes that avoid the variability introduced by the loss of glucose during sample handling. Sample handling during an oral glucose tolerance test may differentially affect the fasting and 2-h postload samples because of the differences in time until processing, thus potentially exaggerating differences between populations in the relationships between fasting and postload glucose concentrations and their relationships to other disease manifestations. Even the reference interval for glucose may be too wide, because it includes the variation produced by the instability of glucose in samples from multiple people. This source of variation may be large, because it reflects both between-sample and between-person variation in glycolysis.

In conclusion, changes in the way blood samples are handled before laboratory measurement of glucose need to be strongly considered. Universal adoption of methods that inhibit glycolysis would be expected to improve the precision and utility of glucose measurements, but it might substantially increase diagnoses of diabetes unless compensatory changes in diagnostic cutpoints were made.

Acknowledgments

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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