

Glucose Measurement: Time for a Gold Standard

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Abstract

There is no internationally recognized reference method for the measurement of blood glucose. The Centers for Disease Control and Prevention (CDC) highlighted the need for standardization some years ago when a project was started. The project objectives were to (1) investigate whether there are significant differences in calibration levels among currently used glucose monitors for home use and (2) develop a reference method for glucose determination. A first study confirmed the assumption that currently used home-use monitors differ significantly and that standardization is necessary in order to minimize variability and to improve patient care. As a reference method, CDC recommended a method based on isotope dilution gas chromatography–mass spectrometry, an assay that has received support from clinical chemists worldwide. CDC initiated a preliminary study to establish the suitability of this method, but then the project came to a halt. It is hoped that CDC, with support from the industry, as well as academic and professional organizations such as the American Association for Clinical Chemistry and International Federation of Clinical Chemistry and Laboratory Medicine, will be able to finalize the project and develop the long-awaited and much needed “gold standard” for glucose measurement.

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When building a house it seems obvious to pay extra attention to the foundation. No matter how nice the architecture is or what fancy building material is used, a stormy night might still cause the building to collapse unless the foundation is solid. Any construction worker will testify to this fact.

The method of calibration has the same fundamental importance for any analytical method. In this age of evidence-based medicine, nothing is more important than the quality of an analytical test. It is commonly believed that two-thirds to three-fourths of the information used for making medical decisions is provided by laboratory tests.

Hence, the test result had better tell the truth about the clinical status of the patient. Yet there is no internationally accepted reference method for the measurement of blood glucose. Several attempts have been made to establish one, but as of today there is still no consensus. At the same time, a lot of time and money continues to be spent on developing new methods and technologies for glucose determination. For the benefit of the patient and diabetes care, as well as industry and regulatory bodies, it is time to once and for all settle this question and build a foundation for glucose measurement that will allow for correct diagnosis, treatment, and standardized/comparable glucose values worldwide.

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Abbreviations: (CDC) Centers for Disease Control and Prevention, (IDGC-MS) isotope dilution gas chromatography–mass spectrometry, (IFCC) International Federation of Clinical Chemistry and Laboratory Medicine, (ISF) interstitial fluid

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The measurement of blood glucose is one of the cornerstones in the screening, diagnosing, and monitoring of diabetes mellitus. Since the introduction of the first glucose point-of-care system in the 1970s, glucose measurement has changed from a solely laboratory-based procedure into a widespread practice in hospital wards, in primary care settings, and among patients with diabetes who perform self-monitoring at home. Today, glucose measurement is the most commonly performed test at the point of care.

There are many methods available for glucose determination, with the majority based on enzymatic reactions involving one of the three enzymes: glucose oxidase, hexokinase, or glucose dehydrogenase.¹ The enzymatic assays are linked to chromogenic reactions that are detected photometrically or to reactions exhibiting changes in electron flow that are measured electrochemically. Specimen types include primarily whole blood (capillary, venous, or arterial) and plasma, but also other sample matrices such as interstitial fluid (ISF).

Do all glucose methods measure the same thing? The answer to that question is “no.” The glucose concentration affecting the analytic system will be determined by whether the method responds to the concentration in the water, in the plasma, in a mixture of plasma and red cells, in a dilution of the blood specimen, or in ISF. This is one of the key challenges of trying to develop a universal reference method or reference material. But even if the same sample type is used, i.e., whole blood or plasma, unacceptable differences have been reported both in the laboratory and at the point of care.²⁻⁴ These differences could be minimized if the same method of calibration was used and would improve the treatment and diagnosis of diabetes.

The Centers for Disease Control and Prevention (CDC) highlighted the need for standardization some years ago when a project was started. The project objectives were to (1) investigate whether there are significant differences in calibration levels among currently used glucose monitors for home use and (2) develop a reference method for glucose determination. Representatives from the industry were invited to participate in the project, and a first study did in fact confirm the assumption that currently used home-use monitors differ significantly and that standardization is necessary in order to minimize variability and to improve patient care.⁵

In this study, five of the most commonly used home monitors were evaluated using two strip lots for each monitor. One of the strip lots was “aged” (less than 25% of shelf life remaining) and the other one “fresh” (more

than 75% of shelf life remaining). Capillary whole blood samples from 93 people with and without diabetes were used, and steps were taken to minimize the effects of preanalytical factors. The average percentage difference between monitor pairs was statistically significant ($p < 0.05$) in more than half of the paired comparisons, with significant differences ranging from 5.7 to 32%. This means that effective treatment is difficult if a person with diabetes changes meter or is using more than one meter, as the reference range is also changing.

In addition to the displayed calibration differences, the study also showed that lot-to-lot variation is a clinically significant concern among home-use monitors. This seems to be a general problem and has been reported by others as well.^{2,4,6,7} Lot-to-lot variation will of course not be solved by standardization, but is a problem that has to be addressed by the manufacturers.

As a reference method, CDC recommended a method based on isotope dilution gas chromatography–mass spectrometry (IDGC-MS), an assay that has received support from clinical chemists worldwide. In this method the sample is diluted very accurately with an isotope of glucose, after which it is prepared and measured in a gas chromatograph with a mass spectrometric detector. The excellent accuracy of IDGC-MS is due mainly to the fact that the only truly critical step is the weighing of the glucose isotope in calibrators and samples.⁸⁻¹¹ CDC initiated a preliminary study to establish the suitability of this method,¹² but then the project came to a halt.

Because of automation and the need for effective logistics, glucose assays are usually performed on plasma in the hospital laboratory. At the point of care, however, it is less convenient to use plasma. In this setting, time is critical. Small sample volumes are sometimes needed and there is a lack of centrifuge facilities, making whole blood the preferred sample type. Glucose is dissolved in water, and as plasma contains more water there is a physiological difference between plasma and whole blood glucose. At normal hematocrit, this difference is approximately 11%. In daily practice, plasma and whole blood glucose are often used interchangeably, despite the physiological difference. In order to avoid clinical misinterpretations, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has recommended that all glucose-measuring devices report in plasma values.¹³ Whole blood values should, according to IFCC, be multiplied by a constant factor of 1.11 to obtain plasma-equivalent glucose values. This conversion has proven to be clinically acceptable, as long as the whole blood value is accurate.¹⁴

For calibration purposes, a universal reference material that works on all glucose measuring systems would be ideal. However, to create such a substrate is an impossible mission, which manufacturers of controls and devices will testify to. The majority of glucose point-of-care devices are made for the analysis of fresh whole blood, and other types of sample material will result in discrepant results due to matrix effects.

Instead of using a reference material, one alternative would be to calibrate a set of reference instruments against IDGC-MS and then use these instruments for calibration of new instruments in production. For optimal calibration, the IDGC-MS should be set up for the same sample type as used by the method to be calibrated; i.e., whole blood methods should be calibrated against the IDGC-MS setup for whole blood, plasma vs plasma, and ISF vs ISF. The reference instruments and disposables used for production should be checked regularly against the IDGC-MS method in order to verify that the calibration level remains stable. This is actually a concept and procedure that has been proven and used successfully by HemoCue AB for years.

An internationally recognized reference method would improve diabetes care dramatically. Today it is not uncommon for a person with diabetes to have two or three meters at home, all calibrated differently and providing different readings. This erodes confidence in the measurements made and makes it more challenging to feel comfortable also in the low-to-normal glycemic range, the requirement for optimal treatment. New technologies for continuous or noninvasive glucose monitoring continue to evolve. A "gold standard" would help regulatory bodies, and the industry, interpret the performance of such systems, which should help expedite the regulatory and approval process.

When discussing the quality of results, preanalytical factors should not be forgotten. Time since last intake of food, exercise, stress, alcohol, and drugs are examples of factors that will influence blood glucose concentrations. Whether the sample is capillary, arterial, or venous must also be known for correct interpretation of the results. Glycolysis, the consumption of glucose by erythrocytes and leukocytes in the sample, is another significant source of error and is therefore one of the most important advantages of (reliable) point-of-care testing. If under standard laboratory conditions high accuracy is required, immediate analysis, centrifugation, storage on ice, or use of protein precipitants becomes a necessity. Knowledge about the potential effects of preanalytical factors is crucial and, if neglected, is something that can invalidate the analysis, regardless of how well the method is calibrated.

The CDC started a very important project a few years ago, a project that hopefully will be finalized. There are still challenges remaining, but with support from the industry, which is key, and continued involvement and support from academic and professional organizations such as the American Association for Clinical Chemistry and IFCC, it should be possible to develop the long-awaited and much needed "gold standard" for glucose measurement.

Disclosures:

Mr. Hagvik is an employee of HemoCue AB.

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