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Approved IFCC recommendation on reporting results for blood glucose[1)]

International Federation of Clinical Chemistry and Laboratory Medicine Scientific Division[2),3)]

Working Group on Selective Electrodes and Point-of-Care Testing (IFCC-SD-WG-SEPOCT)

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Abstract

In current clinical practice, plasma and blood glucose are used interchangeably with a consequent risk of clinical misinterpretation. In human blood, glucose is distributed, like water, between erythrocytes and plasma. The molality of glucose (amount of glucose per unit water mass) is the same throughout the sample, but the concentration is higher in plasma, because the concentration of water and therefore glucose is higher in plasma than in erythrocytes. Different devices for the measurement of glucose may detect and report fundamentally different quantities. Different water concentrations in the calibrator, plasma, and erythrocyte fluid can explain some of the differences. Results for glucose measurements depend on the sample type and on whether the method requires sample dilution or uses biosensors in undiluted samples. If the results are mixed up or used indiscriminately, the differences may exceed the maximum allowable error for glucose determinations for diagnosing and monitoring diabetes mellitus, thus complicating patient treatment. The goal of the International Federation of Clinical Chemistry and Laboratory Medicine, Scientific Division, Working Group on Selective Electrodes and Point of Care Testing (IFCC-SD-WG-SEPOCT) is to reach a global consensus on reporting results. The document recommends reporting the concentration of glucose in plasma (in the unit mmol/L), irrespective of sample type or measurement technique. A constant factor of 1.11 is used to convert concentration in whole blood to the equivalent concentration in plasma. The conversion will provide harmonized results, facilitating the classification and care of patients and leading to fewer therapeutic misjudgments.
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Keywords: activity; biosensors; glucose oxidase; hematocrit; plasma vs. whole blood; standardization; water concentration.

Contents

1. Introduction

The World Health Organization (WHO) and the American Diabetes Association (ADA) define the diagnosis

of diabetes mellitus by at least two measurements of fasting plasma glucose concentration $\geq$7.0 mmol/L. As an alternative, a random venous plasma glucose concentration $\geq$11.1 mmol/L in the presence of symptoms or a 2-h post oral glucose tolerance test result $\geq$11.1 mmol/L suffices to make a definite diagnosis of diabetes mellitus (1, 2). The new classifications of ''impaired fasting glycemia'' have narrower intervals [6.1–6.9 mmol/L (WHO) or 5.6–6.9 mmol/L (ADA)] for venous plasma glucose concentration than the previous fasting interval (5.6–7.7 mmol/L) for classifying normoglycemia and diabetes (3). The narrower diagnostic limits increase the need for reliable results to classify individuals correctly.

Currently, various types of instruments detect and report fundamentally different glucose quantities. Biosensors for glucose are ''direct reading'' when they measure glucose directly, i.e., without prior dilution of the sample. The new generation of direct reading glucose sensors responds to the molality of glucose, which is identical in whole blood and plasma, whereas the concentration of glucose in the two systems is different. Methods requiring high sample dilution produce results equivalent to concentration when calibrated against aqueous standards, because the water concentrations of sample and calibrator are almost identical after dilution.

The original intention of the IFCC-SD WGSE was to make a recommendation for direct reading biosensors in blood gas/electrolyte/metabolite analyzers. However, an isolated recommendation would be meaningless and would not lead to the goal of harmonized results, which requires a consensus on reporting results for all analyzers. Inexpensive instruments with direct reading biosensors are available for self-monitoring or point-of-care testing of glucose (4–6). The clinical chemistry laboratory is expected to perform glucose determinations by direct reading sensors concurrently with other routine instruments that measure substance concentrations in diluted samples.

In current clinical practice, plasma and blood glucose are used interchangeably (7), with a consequent risk of misinterpretation. The two systems are frequently mistaken in the clinical literature, despite an average 11% difference in glucose concentration (plasma > blood). The ADA provides clinical decision limits for the concentration of glucose in venous plasma, but the WHO also provides the concentration of glucose in whole blood (1–3).

With the present use of multiple methods providing results for different quantities, there is a serious risk of clinical misinterpretation. We recommend a constant factor of 1.11 for the conversion between concentration of glucose in blood and the equivalent concentration in plasma, and only reporting the concentration of glucose in plasma to avoid misjudgments. The converted result equals the concentration of glucose in plasma when hematocrit and water concentrations are normal. The recommendation includes point-of-care devices and methods that measure the concentration of glucose in whole blood. The conversion does not eliminate current pre-analytical influences or hematocrit effects, which are specific to certain methods and are summarized elsewhere (4). However, the conversion will provide consistent harmonized results, facilitating the classification and care of patients and leading to fewer therapeutic misjudgments.

The concentration of glucose depends on the sampling site, especially when the patient is not fasting. Therefore, the sampling site must be specified, and information about the sampling site must accompany the result. For testing of glucose tolerance, venous samples are preferred. The conversion factor is only valid for samples from the same sampling site (e.g., venous blood). It does not apply to the calculation of venous plasma glucose concentrations from concentrations of glucose in arterial or capillary blood.

2. Activity and molality of glucose

Biosensors respond to the activity of the analyte in question. The activity of glucose is assumed to be equal to the molality, with a molal activity coefficient equal to 1. Activity (a, dimensionless) is related to the chemical potential ($\mu = \mu_0 + RT\ln a$ in units of kJ/mol). Molality (m, in units of mmol/kg H_2O) is the amount per unit water mass. The relation between m and concentration c (in units of mmol/L) is $m = c/\rho H_2O$, where ρH_2O is the mass concentration of water (in units of kg/L). Calibration of direct reading glucose biosensors with aqueous glucose calibrators without considering the water concentration provides results of ''relative molality'' that are numerically higher than the concentration, but slightly lower than the true molality. Multiplying the results by the ratio of water concentrations in the sample and calibrator converts ''relative molality'' to concentration. The mass concentration of water (kg/L) is 0.71 in average ''normal'' erythrocyte cytoplasm, 0.84 in whole blood/hemolyzed blood, 0.93 in plasma, and 0.99 in aqueous calibrators. ''Normal'' here is defined as a hematocrit of 0.43, and concentrations of proteins and lipids in plasma within the reference interval. The effect of variation within the reference interval is negligible.

Activity is physiologically relevant for determining enzymatic reaction rates, the direction of chemical processes, transport, and binding to receptors. Glucose permeates the erythrocyte membrane quickly by passive transport, facilitated by the erythrocyte glucose transporter, which catalyzes the uniport movement of D-glucose down its concentration gradient. Therefore, the molality of glucose is identical in erythrocytes and plasma. Results obtained by a direct reading glucose biosensor responding to molality are identical for whole blood and its separated plasma.

A new quantity and reference interval for glucose based on molality would add to the present risk of clinical misinterpretation, add to the confusion regarding sample type and analytical methodology, and would not be acceptable in clinical practice. Direct reading glucose biosensors that detect molality of glucose are available on combined blood gas/electrolyte/metabolite analyzers from the major manufac-

turers of these systems. Several point-of-care devices also use direct reading glucose biosensors. Some blood gas/electrolyte/metabolite instruments with direct reading glucose biosensors are calibrated with aqueous calibrators to provide results according to the ''relative molality'' of glucose in the sample. The predicted ratio of results reported by such instruments to those that investigate diluted specimens is 1.18 (0.99/0.84) for normal whole blood and 1.06 (0.99/0.93) for normal plasma, in agreement with results from the literature (8, 9). Unconverted results may be misleading if they are related to established reference intervals and clinical decision limits, and continued use of these instruments without conversion may cause confusion with conventionally determined glucose concentrations.

3. Active concentration of glucose in normal plasma

The converted results based on measured glucose activity and average concentration of water in normal plasma are called the active concentration of glucose in normal plasma to distinguish this from the substance concentration of glucose in actual plasma. We recommend converting and reporting results from all systems and devices using direct reading glucose biosensors as the active concentration of glucose in normal plasma (10) and using the unit mmol/L. This recommendation is in harmony with that of the ADA (2). Another reason for choosing plasma rather than whole blood as the system of reference is its independence from hematocrit. The advantage of direct reading glucose biosensors responding to activity and molality will not be lost. The converted results for direct reading glucose biosensors are proportional to the activity and molality of glucose (contrary to the less physiological substance concentration of glucose), due to the constant factor relationship. The ratio between the active and conventional concentration of glucose in a given plasma sample equals the ratio of the water concentration between average and actual plasma (with a mean of 1.00 and SD of 0.01, if the water concentration is normal). In practice, the two can be considered identical. Subgroups such as neonates or pregnant women may have slightly higher than average plasma water concentrations. A lower than average actual plasma water concentration due to, e.g., hyperlipidemia will lead to a lower conventionally used substance concentration, but has no impact on the active concentration of glucose. The active concentration of glucose is unaffected by changes in water concentration, but the conventionally used concentration will change in proportion to the water concentration.

For the same reason, control material with an assigned glucose concentration must have a normal water concentration of 0.93 kg/L to be valid for quality assessment of direct reading glucose biosensors. Otherwise, the water concentration must be taken into account.

4. Concentration of glucose

Most current photometric methods to measure glucose use enzymatic conversion with NADH or NADPH as coenzymes and absorbance measurements near or at 340 nm. The molar absorptivity permits direct calculation of the glucose concentration after complete reaction.

The concentration can also be determined by a kinetic measurement, comparing the sample to a standard. A kinetic measurement obviates subtraction of the background at the cost of introducing a small positive bias. After 50-fold dilution, the mass concentration of solutes is ~4.0 g/L in the case of whole blood, and less in the case of plasma. The enzymatic reaction rate depends on the substrate activity (or molality). The slightly lower water concentration in a diluted sample compared to a diluted calibrator provides a relatively (slightly) higher enzymatic reaction rate in the diluted sample. Methods that include protein precipitation may also have a positive bias, depending on the degree of dilution and protein concentration. The precipitation creates a non-homogeneous system. The aqueous phase has a higher concentration of glucose than the precipitated protein or the precipitant-containing solution before centrifugation. Despite these (minor) theoretical issues, clinical chemical analyzers measure the glucose concentration (amount of glucose per volume of sample) with sufficient trueness and precision.

Biosensors that require dilution provide results that closely resemble the concentration. These devices report concentration based on the concentration of glucose in the calibrator.

Dilution decreases the concentration of all components except that of the solvent, which approaches the concentration of solvent in the diluent. After dilution at, e.g., a 1:25 ratio, the water concentration of the diluted sample and aqueous calibrator differs by less than 1% at the time of measurement. The consequence is a small positive bias of less than 1% in the reported concentration, depending on the original concentration of the solutes.

5. Plasma vs. whole blood and serum

On a concentration basis (amount of glucose per liter of sample), the glucose concentration in plasma is higher than the glucose concentration in erythrocytes because the water concentration is higher in plasma than in erythrocytes. Unlike direct reading glucose biosensors, methods that use diluted samples produce results that depend on the water concentration of the sample. Therefore, methods requiring sample dilution produce different results for blood (or hemolyzed blood) and the corresponding plasma.

Consider, for example, a blood specimen with a hematocrit (Hct) of 0.43. The water concentration of erythrocytes is ~0.71 kg/L. The water concentration of plasma is ~0.93 kg/L. The water concentration (kg H_2O/L) of the blood specimen must be intermediate

to these extremes $(0.43)\times(0.71)+(1-0.43)\times(0.93)=0.84$. The ratio of the water and, therefore, the glucose concentration between plasma and whole blood is 0.93/0.84, or 1.11. This ratio depends on hematocrit. A decrease in Hct causes an increase in glucose concentration in whole blood and vice versa. When hematocrit is known to be abnormal, the whole blood glucose concentration can be ''hematocrit adjusted'' to a normal hematocrit of 0.43 by multiplication by $0.84/(0.93-0.22\times\text{Hct})$. Unfortunately, some methods may be subject to additional erythrocyte or hemoglobin interference.

Plasma and whole blood glucose concentrations are not interchangeable owing to the difference in glucose concentrations between plasma and whole blood (in contrast to current practice in many institutions). Different reference intervals and clinical decision limits apply. The recommendation here is to report only the concentration of glucose in plasma, irrespective of the material investigated. Glucose and water distribute freely between erythrocytes and plasma, so the molality (but not the concentration) of glucose is identical in erythrocytes and plasma. Therefore, for a given concentration of glucose in plasma, the concentration of glucose in whole blood depends on hematocrit, because erythrocytes have a lower water concentration than plasma. The concentration of glucose in plasma is independent of hematocrit. Glucose activity and concentration are practically proportional in plasma, where the water concentration varies relatively little, but not in whole blood, where hematocrit may vary considerably and confound the relation. The concentration of glucose in plasma (rather than the concentration in whole blood) therefore more closely reflects the activity of glucose. For most purposes, the concentration of glucose in plasma is physiologically more relevant to measure and report than the concentration in whole blood.

The use of serum is discouraged, as the concentration of glucose decreases during its preparation. Likewise, the concentration of glucose in capillary whole blood should not be used as substitute for the concentration in venous plasma (11).

The type of specimen used for glucose measurement has not always received the attention it deserves. For instance, the ADA recommends a maximum allowable CV of 10% at glucose concentrations of 1.7–22 mmol/L and a maximum bias of 15% from a reference method value (12, 13). In other words, the ADA accepts no more than 5% analytical error for glucose determination (13). The systematic 11% difference between normal blood and plasma already exceeds the recommended allowable maximum error. Mistaking or not properly distinguishing the sample type may result in misinterpretation of the result and a wrong diagnosis. According to an editorial (14) by Rainey and Jatlow, reporting whole blood glucose values is anachronistic and comparable to reporting whole blood instead of plasma concentrations of potassium. Manufacturers and clinical chemists should always report the concentration of glucose in plasma to avoid this risk, irrespective of the sample type and method of measurement.

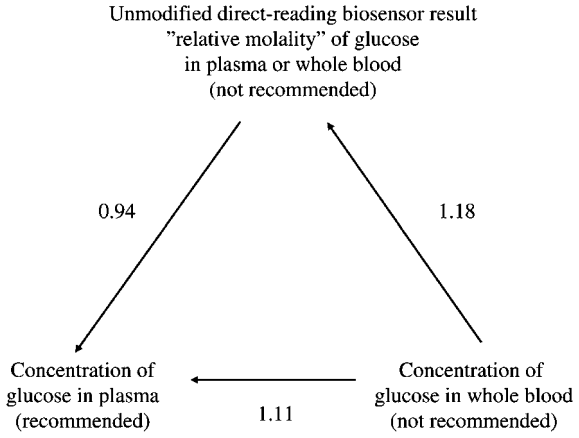

Figure 1 Conversion factors for different quantities of glucose.

6. Conversion of whole blood to plasma equivalent glucose concentration

The present IFCC document recommends using a constant factor of 1.11 for converting the concentration of glucose (Figure 1), based on water concentrations of normal whole blood and of normal plasma. This relationship is supported experimentally (8, 9). Conversion based on measured hematocrit may introduce an additional error (9), besides being less convenient and requiring additional information. Converted (whole blood→plasma) glucose concentrations will have the same dependence on hematocrit as the currently reported whole-blood glucose concentrations. With this convention, all results can be harmonized and reported as the concentration of glucose in plasma. The laboratory, however, must keep information about which sample type and measurement procedures were used.

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