Proposal for standardizing direct-reading biosensors for blood glucose

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Direct-reading glucose biosensors sense molality (glucose per unit water mass) in the sample. With aqueous calibration, a direct-reading glucose biosensor produces higher results in blood and plasma than methods measuring concentration, theoretically by the ratio of water concentrations in calibrator and sample. To confirm this, we measured glucose in 140 blood and 40 plasma samples with the direct-reading glucose sensor in the Chiron Model 860 Blood Gas and Critical Analyte System and with our routine method (ESAT 6660; Eppendorf). The Chiron instrument is calibrated with a 10 mmol/L (180 mg/dL) glucose calibrator (mass concentration of water = 0.99 kg/L). Assuming normal water concentrations of 0.84 and 0.93 kg/L in blood and plasma, respectively, we multiplied results from the Chiron sensor by 0.84/0.99 and 0.93/0.99 to obtain concentrations in blood and plasma. This conversion resulted in agreement of results with our routine method.

An individual conversion based on hematocrit in each whole-blood sample was less satisfactory. To avoid confusion over variously measured and reported glucose results and reference values, we suggest standardization and reporting of whole-blood glucose results as equivalent plasma concentrations. This proposal may be conveniently achieved by using a commercially available reference material for glucose, NIST SRM 965.

Biosensors for blood glucose are called direct-reading sensors when they measure glucose directly without prior dilution of the sample. Such sensors are becoming widely used in modern blood gas/electrolyte/metabolite analyzers. Inexpensive instruments are also available for self-monitoring or near-patient testing of glucose. The clinical chemistry laboratory will, for the foreseeable future, continue to perform glucose determinations by direct-reading sensors used concurrently with other routine instruments.

The various types of instruments used detect and report fundamentally different glucose quantities [1, 2]. Furthermore, the clinical staff in general does not know whether blood or plasma glucose is being measured [3]. Blood and plasma glucose in the clinical literature are frequently mistaken and used interchangeably, despite an average difference of 11% in glucose concentration obtained from methods requiring sample dilution.

The new generation of direct-reading glucose sensors detect the molality of glucose [4]. Therefore, when calibrated against an aqueous standard of a given glucose concentration, the unmodified results for whole blood of normal hematocrit will be 18% higher than the glucose concentration, and those for plasma will be 6% higher. The result will be ~1% lower than the true glucose molality, because the mass concentration of water in the calibration solution is slightly ~1 kg/L. Biosensors requiring sample dilution will produce results similar to concentration, because the water concentrations of sample and calibration solution are almost identical after dilution.

In the present study we describe a direct-reading glucose sensor in a combined blood gas/electrolyte/metabolite analyzer. The reported results by this sensor and by a sensor requiring sample dilution differ in a manner that is predictable when the water concentration of the sample is considered. The molality results from the direct-reading sensor can be multiplied by a factor derived from the ratio of the water concentrations of calibrator and sample to yield the true glucose concentration.

Reference intervals for glucose and glucose tolerance tests are established in clinical practice. With the current practice of using multiple methods, and given the differences between methodologies, there is risk for clinical misinterpretation. We propose that whole-blood results from direct-reading glucose biosensors be standardized and reported as the equivalent concentration of glucose in plasma. A commercially available, serum-based Standard Reference Material for glucose (NIST SRM 965; National Institute for Standards and Technology) adequately serves as a material to achieve this standardization.

The proposed standardization would not eliminate preanalytical influences or hematocrit effects, which may

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be specific to certain methods. It would, however, ensure commutability and allow proper clinical interpretation of glucose results irrespective of method.

**Materials and Methods**

Glucose was measured in 140 blood samples anticoagulated with EDTA-K$_3$ and 40 heparinized plasma samples from patients at Herlev Hospital. In 20 cases, glucose was measured in both whole blood and its separated plasma.

Glucose was measured with an electrochemical biosensor in a Model 860 Blood Gas and Critical Analyte System (Chiron Diagnostics), according to the manufacturer’s instructions. The glucose biosensor uses glucose oxidase and amperometric detection of hydrogen peroxide. To compensate for the presence of interfering substances, e.g., acetaminophen, the sensor incorporates a correcting electrode, which is similar to the measurement electrode but without glucose oxidase [5]. The system alerts the user if the amount of interfering substance detected in a sample produces an error equivalent to 2.5 mmol/L (45 mg/dL) or more in measured glucose. Because of the use of a differential measurement approach (active electrode result minus correcting electrode result), the reported glucose concentration is free from the effects of interfering substances. The glucose sensor is calibrated at glucose concentrations of 0 and 10 mmol/L (180 mg/dL). The mass concentration of water in the latter solution was determined gravimetrically with oven-drying [6] as 0.99 kg/L. The sensor detects molality, but the system reports results relative to the concentration (not molality) of glucose in the calibration solution.

The results from the Chiron instrument were compared with the simultaneously obtained glucose results from our routine method, an ESAT 6660 (Eppendorf-Netheler-Hinz). This system uses a similar glucose biosensor, but requires a 51-fold sample dilution (20 μL of sample in 1 mL of diluent). The dilution stops metabolism of glucose and assures sensor linearity. In this report, the quantity reported by this analyzer is referred to as concentration. Hematocrit was measured with a H*3 device (Bayer Diagnostics).

SRM 965 was obtained from NIST. The material is human serum, provided frozen in flame-sealed glass ampoules. Each unit of SRM 965 has three pairs of ampoules, each ampoule containing 2.00 ± 0.04 mL of serum. The serum in each pair of ampoules has one of three glucose concentrations, certified by the Definitive Method for glucose, isotope dilution mass spectrometry. The water concentration of SRM 965, determined gravimetrically with oven-drying [6], was 0.91 kg/L (mean of two determinations per concentration).

Glucose in SRM 965 was measured with the Chiron instrument. As part of the present study, glucose in SRM 965 was also measured by the hexokinase/glucose-6-phosphate dehydrogenase Reference Method [7] at Johnson & Johnson Clinical Diagnostics, and likewise with a YSI 2300 STAT Blood Glucose Analyzer (Yellow Springs Instruments). The YSI instrument uses a glucose biosensor similar to the ESAT 6660 and performs a 25-fold sample dilution before analysis (25 μL of sample in 600 μL of diluent).

**Results**

No analytical problems occurred during the measurements. One sample from a patient on maintenance hemodialysis at Herlev Hospital caused a warning of a high signal from an interfering substance detected by the correcting electrode. The patient was receiving 4 g of acetaminophen per day, but after we contacted his physician, this unnecessary and potentially harmful medication was stopped.

The Chiron biosensor produced identical glucose readings on blood and its separated plasma by the paired $t$-test [mean difference $= -0.07$ mmol/L ($-1.2$ mg/dL), $n = 20$, $P = 0.11$], suggesting no or negligible interference by erythrocytes. This result is expected because the direct biosensor detects molality (amount of glucose per unit of water mass in the sample), which is identical in whole blood and its separated plasma. The glucose biosensor results were shown to be linear to at least 30 mmol/L (540 mg/dL).

Figure 1 shows the method comparison between the Chiron glucose biosensor and the ESAT 6660 for 140 whole-blood samples, before and after conversion of the direct biosensor results to concentration. The assumed mass concentration of water in whole blood is 0.84 kg/L, whereas the water concentration of the Chiron instrument calibrant is 0.99 kg/L. Accordingly, the unmodified results of the Chiron biosensor were higher than the concentrations by the ESAT 6660, as predicted by the ratio of water concentrations in calibrator/blood = 0.99/0.84 = 1.18. The observed ratio for 140 blood samples was 1.16 ± 0.07 (SD). Therefore, we used a constant factor of 0.85, the inverse of 1.18, to convert the direct biosensor results to concentrations in Fig. 1 (right). This conversion gave accurate results, with no statistically significant difference between instruments by the paired $t$-test [mean difference = 0.03 ± 0.40 mmol/L (0.54 ± 7.2 mg/dL), $P = 0.34$]. An individual conversion based on each sample’s actual hematocrit resulted in a larger bias and SD [0.23 ± 0.46 mmol/L (4.1 ± 8.3 mg/dL), $n = 140$, $P < 0.001$]. The regression equation (Chiron vs ESAT) after the individual conversion was: $y = 1.04x - 0.14$ mmol/L (2.5 mg/dL), $r = 0.998$; $S_{yx} = 0.37$ mmol/L (6.7 mg/dL).

Figure 2 compares the Chiron glucose biosensor results and the ESAT 6660 results for 40 plasma samples, before and after conversion of the direct biosensor results to concentration. The ratio of water concentrations in calibrator/plasma was 0.99/0.93 = 1.06. The observed ratio of glucose results for the 40 plasma samples was 1.06 ± 0.04 (SD). Conversion of the Chiron biosensor data by a factor of 0.94 (the inverse of 1.06) resulted in no statistically significant difference from the ESAT 6660 by the paired $t$-test [mean difference = $-0.03$ ± 0.19 mmol/L ($-0.54$ ± 3.4 mg/dL), $n = 40$, $P = 0.15$].
Measurement of glucose in three concentrations of SRM 965 is shown in Table 1. A unit of material contains a total of six ampoules, one pair for each of three glucose concentrations. We measured glucose in duplicate in each ampoule of a single unit, using both the Chiron instrument and the YSI 2300. Another unit of material was sent to Johnson & Johnson Clinical Diagnostics for measurement by the Reference Method for glucose. Because the measured water concentration in the SRM was 0.91 kg/L, the ratio of the water concentrations (calibrator/sample) $\frac{0.99}{0.91} = 1.08$. Data from the Chiron instrument were multiplied by 0.92 (the inverse of 1.08) to obtain commutable results with the other methods, all of which measure concentration. The data of Table 1 show, as expected, a lowering of the Chiron 860 results after modification.

Comparison of the mean recovery from each method with the NIST-assigned value by use of a $t$-test with unequal variances showed all methods to be equivalent to the NIST assignments (95% confidence) for all concentrations, except for Levels I and III by the Chiron instrument ($P < 0.001$ and 0.005, respectively) and the glucose Reference Method ($P < 0.01$). Compared with the NIST-assigned values, however, the converted Chiron 860 values fell within the range of differences observed in the method comparison for plasma (Fig. 2, right).

**Discussion**

The latest generation of direct-reading glucose biosensors measures molality of glucose (amount of glucose per kilogram of water) in undiluted plasma or whole blood.
The molality of glucose is the physiological relevant quantity (assumed equal to the activity), because it determines the direction of chemical processes, transport, binding to receptors, etc. The glucose molecule permeates the erythrocyte membrane quickly, by passive transport. Therefore, the molality of glucose inside erythrocytes equals that in plasma, providing similar results for blood and plasma glucose when measured with a direct-reading biosensor.

On a concentration basis (quantity of glucose per liter of sample), glucose in plasma is higher than glucose within erythrocytes because the water concentration is higher in plasma than in the erythrocytes. Unlike direct-reading glucose biosensors, sensors that measure diluted samples will produce results that depend on the water concentration of the sample of interest. Therefore, biosensors relying on sample dilution will produce different results for blood and for the corresponding plasma. Consider, e.g., a blood specimen with a normal hematocrit of 43%. The water concentration of erythrocytes is ~0.71 kg/L. The water concentration of plasma is ~0.93 kg/L. Therefore, the water concentration (kg/L) of the blood specimen is:

$$\left(0.43 \times 0.71\right) + \left(1 - 0.43\right) \times 0.93 = 0.84$$

Biosensors that require dilution before measurement will underestimate molality, because when whole blood is diluted, e.g., in a 1:25 ratio, the water is diluted 0.84: (25 × 0.99), or 1:29.5. When plasma is the sample, the water is diluted 0.93: (25 × 0.99), or 1:26.6, providing 11% higher results for plasma than blood. The water of the aqueous calibration solution is diluted 0.99: (25 × 0.99), or 1:25. Biosensors that require dilution are usually calibrated in terms of the concentration (not molality) of glucose in the calibration solutions. Because of dilution, the water concentration of sample and calibrant will differ by <1% at the time of measurement, resulting in a small positive bias of <1% in the reported concentration. When direct-reading glucose biosensors are calibrated on a concentration basis, the predicted ratio of results for the two methods (direct/diluted) will be 0.99/0.84 = 1.18, in good agreement with the data observed for the blood samples of Fig. 1. Likewise, for plasma, with an assumed water concentration of 0.93 kg/L, the predicted ratio of results will be 1.06, in good agreement with the data of Fig. 2.

Direct-reading glucose biosensors are now offered on combined blood gas/electrolyte/metabolite analyzers from all major manufacturers of these systems. As these systems continue to be further utilized, there will be an increased risk of misinterpretation of variously measured and reported blood glucose results, when compared with established reference intervals. Furthermore, a new quantity and reference interval based on molality would probably not be accepted in clinical practice. We thus propose that blood glucose results from systems that use direct-reading glucose biosensors be converted and reported as the equivalent concentration of glucose in normal plasma. The molality of glucose is physiologically more relevant than the concentration, and the advantage of the direct-reading glucose biosensors detecting molality will not be lost by the suggested conversion. The results will be proportional to molality, because the conversion factor is a constant.

The SRM 965 from NIST, with values assigned by the Definitive Method for glucose, provides a convenient way to achieve the proposed standardization, as indicated in Table 1. The assigned concentrations of SRM 965 should be adjusted 2% upwards for use with direct-reading glucose biosensors, because of the slightly lower than normal water concentration in the SRM. The water-corrected SRM data from Table 1 for the Chiron 860 still show a small, but statistically significant, difference from the NIST-assigned values at 95% confidence for the Level I and III concentrations. These residual differences may

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<th>Table 1. Measurement of glucose in NIST SRM 965 with a direct glucose biosensor (Chiron 860) and methods requiring sample dilution.</th>
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* Water concentration of SRM 965 = 0.91 kg/L.
* 95% confidence interval.
* Mean of two measurements in each of two ampoules (n = 4).
result from other instrument influences, e.g., fluidics, on sensor recovery. The proposed standardization with SRM 965 would remove these differences as well. Adjustment of whole-blood data could be achieved by a simple slope and intercept correction of the direct biosensor results, by use of factors derived from a linear regression method comparison of the biosensor results for the SRM with the NIST-assigned values. This approach resembles the practice for adjustment of direct ion-selective electrode results for sodium and potassium to those of the flame photometric Reference Method, as proposed and described in detail in NCCLS document C29-A [8].

Methods involving sample dilution have been used to establish reference intervals for glucose and glucose tolerance tests. Systems that require sample dilution before measurement will provide results for plasma 11% higher than those for blood of normal hematocrit. The difference depends on the water concentration and therefore the hematocrit of the sample. This hematocrit effect exists for all methods that require sample dilution, regardless of the measurement technology used. A 10% change in hematocrit corresponds to 2.6% change in measured glucose in the opposite direction because of the difference in water concentrations between plasma and erythrocytes. Unfortunately, some methods may have additional erythrocyte or hemoglobin interferences [1]. We support the recommendation of one manufacturer of a blood glucose analyzer requiring sample dilution for converting whole-blood glucose concentrations to their plasma equivalent values [9].

The type of specimen analyzed has not always received the attention it deserves. For instance, the American Diabetes Association has recommended a maximum error of 10%, from a reference measurement value, for future instruments [10]. The systematic 11% difference between normal blood and plasma alone exceeds the recommended maximum error. According to WHO [11], the lower limit for fasting blood glucose in diabetes is 6.7 mmol/L (120 mg/dL). The upper reference limit for fasting plasma glucose is 6.1 mmol/L (110 mg/dL). Mistaking sample type or reference interval could result in a wrong diagnosis. To avoid this risk, we suggest that all glucose results be converted to plasma concentrations, irrespective of the sample type analyzed and the method of measurement. This approach could also be extended to glucose measurement systems for self-monitoring or near-patient testing. In fact, one glucose meter for bedside monitoring has been designed to measure whole blood but to report a plasma equivalent result, to reduce possible confusion when comparing glucose meter and laboratory glucose values [12].

As the data presented here indicate, in the case of the new direct-reading biosensors for glucose, a standardized conversion derived from the water concentrations in normal blood and plasma can be used. For an individual conversion to result in a more accurate estimate of concentration, a true measurement of water concentration of each sample would be needed. Measurement of hematocrit alone appears to be insufficient and introduces additional sources of error, besides being less convenient. We found that a standardized conversion was best, resulting in better agreement when molality was converted to glucose concentration for patients' samples. The same may apply for the suggested whole blood to plasma conversion for systems that require sample dilution.

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References