HemoCue: Evaluation of a Portable Photometric System for Determining Glucose in Whole Blood

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We assessed the HemoCue system for measuring glucose in 5 µL of whole blood. A glucose dehydrogenase-based reaction is used with dried reagents contained in disposable microcuvettes, which are filled with blood by capillary action. Automated hexokinase and YSI 23AM glucose analyzer methods were used for comparison. Overall imprecision (CV) was better than 4.5%, with no significant differences in results between the three different HemoCue photometers and four batches of microcuvettes. Regression slopes (± SE) were 0.947 (0.011) with the YSI and 0.966 (0.015) with the hexokinase method. Analytical recovery of added glucose was 101–106%, and the system functioned with hematocrits up to 0.65. Bilirubin up to 453 µmol/L did not interfere, but high concentrations of endogenous (>3 mmol/L) and exogenous triglycerides gave positive interference. The system proved stable and robust under a wide range of storage and handling conditions; performance was impaired only at high ambient temperature (37 °C). We conclude that the HemoCue system should prove useful for glucose measurement; further testing outside the laboratory is warranted.

Additional Keyphrases: enzymatic methods · point of care testing

Blood glucose measurement is one of the most ubiquitous biochemical tests, being performed in laboratories, in wards, in clinics, in the field, in doctors' offices, and in the home (1). The trend in glucose measurement has been towards the use of enzyme-based methods with greater specificity and the introduction of easy-to-use analytical systems, often with disposable components. However, the small photometers developed in recent years, with their associated glucose oxidase reagent-impregnated test strips and pocket-sized electrochemical sensors, have not yet matched the best laboratory-based wet chemistry methods for precision and accuracy (2, 3).

The HemoCue (HemoCue AB, Angelholm, Sweden) is a portable photometric system into which whole blood is introduced by capillary action. After lysis of the cells within the cuvette, whole-blood glucose concentrations are measured by use of a coupled glucose dehydrogenase reaction. The reaction is performed in a unique disposable reaction microcuvette. We examined the HemoCue system to assess its potential utility in a diagnostic laboratory and in field-based epidemiological studies.

Materials and Methods

HemoCue system. The photometer is precalibrated by the manufacturer, using whole blood with a glucose concentration of ~10 mmol/L, this concentration having been established with a wet chemical glucose dehydrogenase method and cross-referenced against a glucose oxidase/ H2O2 polarographic sensor. The analyst may check this preset calibration by using an optical interference filter in the form of a control cuvette. Analytical microcuvettes containing the dried enzymes and saponin are supplied in sealed desiccated containers. The cuvette design gives a light path of 0.16 mm, thereby facilitating measurements of 5 µL of undiluted blood, which fills the cuvette chamber by capillary action. Introduction of the cuvette into the photometer within 40 s initiates bichromatic monitoring at 680/840 nm. The reactions are as follows (4):

Glucose dehydrogenase

\[
\text{Glucose} + \text{NAD}^+ \rightarrow \text{gluconolactone} + \text{NADH}
\]

Diaphorase

\[
\text{NADH} + \text{MTT (tetrazolium salt)} \rightarrow \text{formazan} + \text{NAD}^+
\]

where MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide.

Timing of the reaction endpoint, and therefore the display of the results, is glucose concentration dependent and varies from <90 s for values up to ~8 mmol/L to 240 s at 22.2 mmol/L, the upper end of the instrument’s functional range.

Comparison systems. We compared the performance of the HemoCue with two other enzymatic methods, the Cobas-Bio centrifugal analyzer (Roche Diagnostics, Welwyn Garden City, UK), using an in-house hexokinase (EC 2.7.1.1) method (5), and the YSI Model 23AM (Yellow Springs Instrument Co., Yellow Springs, OH), based on a glucose oxidase (EC 1.1.3.4)-impregnated membrane on a polarographic electrode. The hexokinase method was standardized with a 50 mmol/L aqueous glucose standard (Merck Ltd., Poole, UK) and the YSI with a 10 mmol/L aqueous glucose standard (Cladon Scientific, Aldershot, UK).

Blood samples and quality control. Venous blood samples were used in the evaluation and were collected into fluoride oxalate. These were analyzed without further pretreatment, except that in the hexokinase method 1 mL of blood was mixed with 5 mL of ice-cold perchloric acid (50 g/L), and centrifuged (2500 × g), and the supernatant was analyzed. For quality-control monitoring throughout the study we used three concentrations of Seronorm (Nycomed AS, Oslo, Norway), which has a serum-based matrix. No
reliable whole blood or similar substitute control material is currently commercially available.

Imprecision. Within-run performance was measured by replicate analysis (n = 20) of quality-control materials. Between-run characteristics were monitored over the 1 mo of the study with the same materials (n = 30). We also assessed the performance of the photometer, using the manufacturer's control cuvette (n = 30). Interopera-
tor reproducibility of results from the HemoCue and its ease of use were further assessed by having measurements made by users with widely different extents of analytical experience. Comparability of results obtained with different HemoCue photometers (n = 3) and cuvette batches (n = 4) was also investigated.

Linearity/recovery. The linearity of the glucose chemistry within its functional range (up to 22.2 mmol/L) was assessed by adding a constant volume of various aqueous glucose standards to equal volumes of previously analyzed blood samples. The measured differences were then related to the added glucose to determine percentage recovery.

Interferences. The influence of hematocrit over the range 0.35–0.65 was assessed at normoglycemic and hyperglycemic conditions. To examine the effects of bilirubin and triglycerides, we used samples with normal and pathological concentrations of these potential interferents. The effect of exogenous triglyceride was examined by adding small volumes of 20% Intralipid (Kabi Vitrum, Uxbridge, UK) to whole blood and to an aqueous glucose standard as control, to give a range of triglyceride concentrations up to ~9 mmol/L. The effect of ascorbic acid was investigated by adding it to whole blood in concentrations up to 0.8 g/L.

System robustness. We investigated the viability of analytical microcuvettes under a variety of adverse storage conditions that might be experienced in field use, including prolonged exposure to the atmosphere without desiccant and exposure to temperature extremes of −20 and 37 °C. The manufacturer's claims for the robustness of the photometer were further assessed by analyzing a quality-control material after repeatedly (n = 5) dropping the instrument onto the laboratory floor from bench height. Photometer performance when plugged into the electrical system and when operated on battery-only power was compared, as was the functioning of the system as a whole at ambient laboratory temperature of 23 °C and also at 30 and 37 °C.

Statistics. Data analysis was performed with the PC-based package Statgraphics V2.6 (STSC Inc., Rockville, MD).

Results

Within-run imprecision for various categories of users is shown in Table 1. Between-run imprecision CVs for the same materials in experienced hands over 30 working days were 4.0% at a glucose concentration of 4.5 mmol/L, 3.8% at 9.3 mmol/L, and 3.9% at 17.6 mmol/L. Replicate measurements (n = 30) with a control cuvette gave a CV of 0.6%.

A series of 30 blood samples (glucose concentrations 1.8–17.2 mmol/L) was used to compare three photometers and four batches of cuvettes. After logarithmic transformation of the resulting data, multi-factor analysis of variance (ANOVA) showed that, although the main source of variation was attributable to the photometers, the glucose results from all combinations of photometers and microcuvettes tested were homogeneous, there being no statistically significant differences in these combinations at the 95% confidence level.

The relationships between the HemoCue glucose results for 123 subjects and those obtained with the comparison systems gave the following intermethod regression equations (± SE estimates):

\[
\text{Hemoglobin} = 0.964 (±0.013) \text{YSI} + 0.167 (±0.584) \text{mmol/L} \\
\text{HemoCue} = 0.947 (±0.011) \text{YSI} + 0.295 (±0.490) \text{mmol/L} \\
\text{HemoCue} = 0.966 (±0.015) \text{hexokinase} + 0.234 (±0.646) \text{mmol/L}
\]

Analytical recovery of added glucose within the functional range of the system was 101–106%. Ascorbic acid gave a positive interference in the HemoCue measurement of blood glucose of 0.15 mmol/L per 0.1 g/L ascorbate concentration. Bilirubin in the range 87–453 \(\mu\text{mol/L}\) did not give any significant interference.

Comparison studies on the effects of endogenous triglyceride (0.8–8.2 mmol/L) on glucose determinations with the HemoCue, YSI, and hexokinase methods are presented as difference plots (6) in Figure 1. Significance testing of these plot slopes (95% confidence level) indicated that only differences between the YSI and HemoCue results were correlated significantly (P < 0.05) with triglyceride concentration; however, these differences would not be clinically significant at triglyceride concentrations <3 mmol/L. Addition of exogenous triglyceride to normoglycemic blood samples with endogenous lipid concentrations of ~1 mmol/L showed a marked positive interference in the HemoCue system but was not evident for the YSI. Average glucose increases of 40% and 105% resulted when the basal

| Table 1. Effect of Analyst's Skill Level on Within-Run HemoCue Performance Statistics |
|-----------------------------|-----------------------------|-----------------------------|
| Q1                         | Experienced | Inexperienced | Nonlab. worker |
| Mean, mmol/L               | 4.3         | 4.2           | 4.0           |
| SD, mmol/L                 | 0.09        | 0.16          | 0.18          |
| CV, %                      | 2.1         | 3.8           | 4.5           |
| Q2                         | Mean, mmol/L | 9.1           | 9.2           |
| SD, mmol/L                 | 0.20        | 0.21          | 0.23          |
| CV, %                      | 2.2         | 2.3           | 2.5           |
| Q3                         | Mean, mmol/L | 18.0          | 17.4          | 17.5          |
| SD, mmol/L                 | 0.36        | 0.40          | 0.42          |
| CV, %                      | 2.0         | 2.3           | 2.4           |

n = 20 in all cases.
triglyceride concentration was increased by 1.28 and 7.7 mmol/L, respectively. In a control experiment, results increased by 30% when an aqueous glucose solution was supplemented with lipid at 1.28 mmol/L.

Increasing the hematocrit from 0.35 to 0.65 did not affect the glucose results. At hematocrits >0.65, the auto-filling capillary effect was insufficient to permit blood to enter the cuvette.

Varying the conditions under which the analytical microcuvettes were stored affected the results obtained with a quality-control serum. Table 2 shows the mean percentage changes for replicate determinations from a baseline value of 6.3 mmol/L obtained with similar microcuvettes stored according to the manufacturer's specifications (i.e., sealed at 4 °C). Analyzing blood samples (n = 19) with glucose concentrations of 2.9–20.6 mmol/L after both photometer and microcuvettes were stored at 23, 30, or 37 °C, to simulate field use of the system, showed a significant change in HemoCue glucose values only for the comparison between 37 and 23 °C (mean difference ± SE = 0.78 ± 0.07 mmol/L).

When we dropped the photometer onto the laboratory floor between measurements, the range of mean glucose results was 5.4–5.6 mmol/L (n = 5 in duplicate):

Measuring blood glucose concentrations (n = 12) in the range 4.8–9.3 mmol/L with the photometer on main power and repeating the measurement on battery power gave results that were not significantly different from each other.

Discussion

Desirable limits of performance reproducibility (CV) for blood glucose measurements have been variously defined to be an ideal of 2.2% (7) or, for outside-the-laboratory testing, <5.0% (8). The latter was achieved by all users of the HemoCue, thereby confirming its ease of use. Few, if any, current glucose methods are capable of the rigorous performance set as a goal by Fraser (7).

We found the photometer to be both portable and durable. The functional glucose range of the system was consistent with that of many other portable and laboratory glucose analyzers and only slightly less extensive than for the comparison methods used. However, the reaction time of 1.5 to 4 min is longer than for many other methods and could hamper its usefulness in a busy clinic or screening program. Reproducibility over the period of the study was good and results by the HemoCue, despite its less direct mode of calibration, agreed closely with those of the comparison methods, which were standardized with primary materials. Furthermore, results of analyses performed with different HemoCue instruments and cuvette batches were comparable within the test laboratory environment. How-

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<th>Storage conditions</th>
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<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>4 °C/sealed</td>
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<tr>
<td>23 °C/sealed</td>
<td>1.6</td>
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* Percentage change from optimum (4 °C/sealed) baseline determined with control material with a glucose concentration of 6.3 mmol/L.

Table 2. Influence of Microcuvette Storage Conditions on Assay Performance

Fig. 1. Intermethod blood glucose differences in relation to increasing concentrations of endogenous triglyceride

Slopes ± SE: (a) YSI vs hexokinase, 0.015 ± 0.059; (b) YSI vs HemoCue, 0.073 ± 0.031; (c) hexokinase vs HemoCue, 0.058 ± 0.066
ever, the fact that the user cannot readily recalibrate the system may be a disadvantage when results are to be compared with those of a laboratory glucose analyzer not calibrated with a primary standard.

Performance also depends on proper storage of the microcuvettes. Although these could be stored at various temperatures—from freezer to 23 °C—for 2 wk, longer storage was suitable only at the recommended 4 °C. Moreover, we observed that a system functioning at 37 °C could yield glucose results almost 1 mmol/L higher than if used in more temperate laboratory conditions—which is not consistent with the manufacturer’s claims. Differences of the magnitude shown at 37 °C could lead to misclassification of subjects as having impaired glucose tolerance or diabetes, according to the WHO 75-g oral glucose tolerance test criteria for epidemiological studies (9). Although it is now recommended that screening programs be based on laboratory-determined results, this is impractical in many settings in developing countries. We have used YSI analyzers in rural East Africa with proper quality-assurance checks (10), but a system such as the HemoCue would be both cheaper and more practical for use there.

The interfering effects of endogenous triglyceride are less than for the exogenous material, for which the effects are substantial. This interference could compromise accuracy of measurement in some patients, in view of the association of hyperlipidemia with diabetes. The greater interference from exogenous lipid may well reflect chemical interference from the emulsifiers included in the Intralipid preparation. Such agents are present in many other similar parenteral preparations used in acutely ill patients, whose nutritional maintenance may be based solely on such formulations and in whom serious glucose measurement errors would be likely to occur with the HemoCue. Although ascorbate interference will be significant only with high dosages, this finding provides a further example of chemical interference in the HemoCue method, probably through enzymatic production of formazan. The primary enzyme-mediated reaction is highly specific for glucose, but the lesser specificity of the coupled reaction compromises the accuracy of glucose measurements in these special cases.

In conclusion, although the HemoCue glucose system could be operated satisfactorily in a controlled laboratory environment, its use where much higher ambient temperatures prevail will require extra care. Interference problems are a significant impediment to use of the HemoCue in acute care, and cuvette-filling difficulties with polycythemic blood would have implications for its use in neonatal practice but will not be a problem in routine use. With the above caveats, the HemoCue should prove useful for glucose measurement, and further testing outside the laboratory is warranted.

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References