Accurate and precise isotope dilution mass spectrometry method for determining glucose in whole blood

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An accurate and precise method to determine glucose concentration in whole blood is presented. The method, based on isotope dilution gas chromatography–mass spectrometry (ID GC-MS), was developed to be used as a Reference Method for determining glucose concentration in capillary or venous whole blood. Blood samples and standards are pipetted manually with “microcap” micropipettes, which makes it possible to collect samples even at the patient’s bedside. Glucose is quantified as its aldononitrile pentaacetate. [13C6]Glucose is used as an internal standard. Assay of Seronorm and Pathonorm L and H controls by ID GC-MS gave within-run CVs of 0.66%, 0.96%, and 0.92%, respectively. For whole blood with glucose concentrations in the low, normal, and high ranges, the within-run CVs were 1.27%, 0.91%, and 0.78%, respectively. The between-run CV for glucose calculated from 36 separate single analyses of Seronorm was 1.44%. In an accuracy assessment test of the HemoCue blood glucose analyzer, 140 capillary blood samples were measured in parallel after split-sampling. For all samples the HemoCue analyzer results had a mean bias of +2.0% compared with the ID GC-MS results.

INDEXING TERMS: gas chromatography–mass spectrometry • quality control • near-patient analysis • HemoCue glucose meter compared

During the past 10 years, there has been a fast growth of new glucose meters for monitoring blood glucose. These instruments are frequently used at home, at the physician’s office, and at diabetic clinics. Although they are generally checked with a routine clinical laboratory method for glucose determination, there is no common basis for comparing the accuracy and precision of these instruments [1]. The importance of reliable methods for self-monitoring of blood glucose is obvious from the results of the Diabetes Control and Complications Trial [2].

The technique of isotope dilution gas chromatography–mass spectrometry (ID GC-MS) has been used in many efforts to establish Reference Methods or Definitive Methods for glucose in serum [3–6]. This technique has also been used since the mid-1970s by the US National Institute of Standards and Technology (NIST) in their development of such methods for glucose and other endogenous analytes in serum [7, 8]. Pioneers in the field of establishing Reference Methods and Definitive Methods were Björkhem et al. [9], who developed an ID GC-MS method for glucose in serum so as to evaluate routine methods for glucose determination.

All the currently published Reference Methods and Definitive Methods for glucose are applicable to measurement of glucose in serum and not in whole blood [10]. The Reference Methods and Definitive Methods used by the NIST and others [3–5] for determination of glucose in serum all have very high accuracy and precision. This high accuracy and precision is in part achieved by the way the samples and standards are added in the methods. Serum and standards are measured gravimetrically, and the serum samples are bracketed by internal standard and standard samples having a concentration of glucose just above and below that of the serum sample. This requires preliminary quantification of glucose in the sample to find a proper concentration for the internal standard and the glucose standard.

We describe here an ID GC-MS method for glucose in whole blood. The derivatization of glucose is essentially performed as described by Stökl and Reinauer [5] and Magni et al. [6]. The blood and standard solutions are sampled volumetrically. Instead of the bracketing technique presented by Cohen et al. [11], we use six standard solutions of glucose, ranging from 1.0 to 20.0 mmol/L,
and a fixed concentration of the internal standard, $[^{13}\text{C}_6]\text{glucose}$.

We also present the results of an accuracy assessment of the HemoCue blood glucose analyzer in comparison with the ID GC-MS method as a Reference Method. The glucose was measured by split-sample technique in capillary blood of 67 diabetic patients and 62 nondiabetic persons.

**Materials and Methods**

**Apparatus.** GC-MS. The instrumentation consisted of an HP 5890 Series II gas chromatograph with an HP 7673 autosampler and a column inserted directly into the ion source of an HP MSD 5970 mass spectrometer (Hewlett-Packard, Palo Alto, CA). The gas chromatograph was equipped with a 25 m $\times$ 0.25 mm CP-Sil-13 CB WCOT fused-silica column coated with a film 0.20 $\mu$m thick (Chrompack International BV, Middelburg, The Netherlands). The carrier gas was helium at a head pressure of 45 kPa. The injector was used in the splitless mode and its temperature was kept at 270 °C. The oven temperature was ramped from 80 °C to 300 °C at 20 °C/min. In these conditions, the derivatives of glucose and $[^{13}\text{C}_6]\text{glucose}$ were eluted after $\sim$11.4 min. The transfer line of the column to the ion source was kept at 260 °C. The mass spectrometer was calibrated with perfluorotributylamine through the “Standard Autotune” program of the “Chem Station” software (Hewlett-Packard). The analyses were carried out by selected ion monitoring, with acquisition of ions at $m/z$ 314.0 and 242.1 for unlabeled glucose and ions at $m/z$ 319.0 and 246.1 for $^{13}\text{C}$-labeled glucose. The acquisitions for all four ions together were done during scan cycles of 0.17 s. These conditions meant that $\sim$30 scans were performed during elution of the glucose peak. The $m/z$ 314.0 ion comes from the loss of CH$_2$OC(O)CH$_3$ from the molecular ion; $m/z$ 242.1 originates from the further loss of CHO(C)CH$_3$. The corresponding ions of $[^{13}\text{C}_6]\text{glucose}$ contain five and four atoms of $^{13}\text{C}$, respectively.

HemoCue blood glucose analyzer. The instrument and the cuvettes came from HemoCue (Ångelholm, Sweden).

**Sampling equipment.** Capillary blood from diabetics and nondiabetics was collected with plain 50-μL Vitrex “microcap” micropipettes (Modulohm 1/S, Herlev, Denmark). The internal standard solution, 200 μL, was manually delivered with a 40–200-μL adjustable micropipette (Finnpipette, Labsystems OY, Helsinki, Finland).

**Reagents.** Standard solutions and controls. We prepared standard solutions from glucose (anhydrous, analytical reagent; Mallinckrodt Chemicals, St. Louis, MO) or glucose Standard Reference Material (SRM 917a; NIST, Gaithersburg, MD). After drying the substance for 24 h at 90 °C, we stored it over a drying agent. Deionized water was added to 360.3 mg of glucose [as weighed with a semimicro balance (Model MC210P; Sartorius, Göttingen, Germany), which has a readability of 0.01 mg and a specified maximal inaccuracy of 0.03% when weighing 360.3 mg], to give a final volume of 100 mL; the glucose concentration of the solution was 20.0 mmol/L. Using volumetric pipettes and volumetric flasks, we further diluted this solution to 10.0, 6.0, 4.0, 2.0, and 1.0 mmol/L, as follows. From the 20.0 mmol/L standard we took 50.0 mL and diluted it to 100 mL with water, yielding a glucose concentration of 10.0 mmol/L. From the 10.0 mmol/L solution we took 30.0, 20.0, and 10.0 mL, diluting each to 50.0 mL to yield glucose standard solutions of 6.0, 4.0, and 2.0 mmol/L, respectively. The 1.0 mmol/L standard was prepared by taking 10.0 mL of the 10.0 mmol/L glucose solution and diluting it to 100 mL with water. These standard solutions were divided into portions and stored at $-20 ^\circ$C; repeated assays after various storage periods showed them to be stable for at least 6 months under these conditions.

To determine the between-run CV, we analyzed Se-ronorm (Nycemed Pharma AS, Oslo, Norway; lot no. 182) as a control with each series of blood samples. For the low and high range of glucose concentration, Pathonorm L and Pathonorm H (Nycemed; lot nos. 010026 and 503418, respectively) were analyzed. To assess the accuracy of the method, we used SRM 909b Level I from NIST.

**Internal standard solution.** $[^{13}\text{C}_6]\text{Glucose}$ (Cambridge Isotope Labs., Andover, MA) was used as an internal standard. The solution was prepared by weighing 9.31 mg of $[^{13}\text{C}_6]\text{glucose}$ and adding deionized water to a final volume of 50 mL, giving a concentration of 1.0 mmol/L. The solution was stored at $-20 ^\circ$C in 5-mL portions.

**Chemicals.** All chemicals were of analytical grade. Pyridine was from May & Baker (Dagenham, UK) and acetic anhydride, methanol, and hydroxylamine hydrochloride were purchased from Fluka Chemie (Buchs, Switzerland). The pyridine was distilled before use and stored over KOH pellets.

**Analytical Procedure.** Blood samples were collected from patients with diabetes mellitus and from apparently healthy nondiabetic individuals. The sampling was approved by the Committee for Medical Ethics at the University Hospital of Linköping and was conducted in accordance with the rules of the Committee. Capillary blood (50 μL) was taken with a microcap and immediately transferred to a tube containing 200 μL of $[^{13}\text{C}_6]\text{glucose}$, 1.0 mmol/L. The microcap was washed five times with the internal standard solution and the tube was thoroughly shaken. The sample was left on the bench for 1–2 h, and 1 mL of methanol was added for deproteinization. After mixing the sample, we let it stand for 30–45 min and then centrifuged it for 10 min at 1000g. A portion (0.5 mL) of the methanol/water phase
was taken to dryness under a stream of nitrogen on a heating block at 50 °C. The glucose and [\textsuperscript{13}C\textsubscript{6}]glucose in the sample were then converted to aldononitrile derivatives by adding 150 μL of 0.2 mol/L hydroxylamine hydrochloride solution in pyridine and heating at 90 °C for 40 min. After cooling, the derivatives were acetylated by addition of 200 μL of acetic anhydride, followed by heating at 90 °C for 60 min. The samples were then evaporated just to dryness under nitrogen (at 50 °C), and the residues were dissolved in 0.5 mL of chloroform. Before the GC-MS analysis, 15 μL of the solution was diluted with 135 μL of chloroform in small glass vials that were then capped with polytetrafluoroethylene septum caps. By dissolving the samples in 0.5 mL of chloroform and diluting the samples an additional 10-fold in chloroform, we could detect no deterioration of peak shape from eventual residual hydroxylamine, not even after several hundred injections. The vials were loaded into the autosampler, which was programed to inject 1 μL of each sample into the gas chromatograph. The standards and the controls were treated the same as the blood samples. A calibration curve was produced and a control sample was included in every batch of blood samples measured. All samples, standards, and controls were measured singly, and results were quantified by measuring the peak areas for m/z 314.0 and 242.1 for glucose and those for m/z 319.0 and 246.1 for [\textsuperscript{13}C\textsubscript{6}]glucose. The m/z 242.1 and 246.1 ions were also used as “qualifier ions” in the ChemStation software. The qualifier ions served to confirm the identity of the glucose and [\textsuperscript{13}C\textsubscript{6}]glucose derivatives. The concentration of glucose in an unknown sample was calculated from a linear regression fit of the peak area ratios (m/z 314.0 + 242.1)/(m/z 319.0 + 246.1).

**OTHER STUDIES**

**Influence of glycolysis.** The effect of the glycolysis in the analysis procedure was tested on three different occasions by collecting into EDTA K\textsubscript{3}-containing tubes a blood sample from nondiabetic individuals. Blood aliquots were taken from the tubes with 50-μL microcaps and transferred to seven small plastic tubes containing 200 μL of internal standard solution. After the microcaps were washed five times in the internal standard solution, each sample in the plastic tubes was mixed. Methanol was then added to the tubes after 5, 15, 30, 60, 120, 180, or 240 min. The samples were then analyzed as described in the preceding section.

**Estimation of accuracy, precision, and specificity.** The accuracy of the method was assessed by measuring glucose in SRM 909b Level I with five analyses at two different occasions with use of both glucose from Mallinckrodt and the NIST SRM 917b as standard material. Within-run imprecision was determined by multiple analyses of whole blood, Seronorm, Pathonorm L, and Pathonorm H in the same series. The between-run imprecision was calculated from independent single analyses of the control samples on different days. Seronorm was analyzed as a single sample on 36 different days within a period of 10 months; Pathonorm L and Pathonorm H were measured as single samples on 19 and 21 different days, respectively. The within-run and between-run imprecision was determined so as to include all parts of the analysis, i.e., sample collection, sample preparation, and quantification of glucose with the GC-MS instrument. Assay specificity was demonstrated by analyzing a sample of EDTA K\textsubscript{3}-treated blood that had been gently mixed for a little more than 3 days. During this time, all the glucose was consumed by glycolysis, so that any interfering substances present should be disclosed in the chromatogram. The imprecision in the two pipetting steps in the method was determined by performing multiple weighings of the 50-μL microcaps filled with water and by weighing the amount of water delivered by the 40–200-μL adjustable micropipette.

**Linearity.** We used a standard curve over the 1.0–20.0 mmol/L concentration range and checked the linearity by linear regression fit.

**HemoCue/ID GC-MS comparison.** The HemoCue blood glucose analyzer is a bedside or near-patient analyzer with dedicated cuvettes; glucose dehydrogenase, mutarotase, and diaphorase act on the sample in connection with a color-generating reagent. The glucose is measured after the erythrocytes have been lysed in the cuvette. The analytical principle was described by Banauch et al. [12]. The recommended measurement range for the HemoCue blood glucose analyzer is 0–22.2 mmol/L. The HemoCue analyzer is calibrated by the manufacturer against an automated glucose dehydrogenase method and a Yellow Springs Instruments glucose analyzer (YSI Ltd., Hampshire, UK). Each individual analyzer is equipped with a control cuvette to assure the stability of the calibration. For an analysis to be acceptable, the result for the control cuvette may not deviate >± 0.3 mmol/L from the target value. The effect of hematocrit on the HemoCue blood glucose analyses has been studied by Wiener [13], who saw no appreciable effect from variations in the hematocrit.

In the comparison study, the HemoCue instrument with disposable cuvettes was carried to the patients, as were small plastic tubes containing 200 μL of [\textsuperscript{13}C\textsubscript{6}]glucose, 1.0 mmol/L (used as sampling tubes for the ID GC-MS method). Capillary blood was collected into the HemoCue cuvette, which was inserted into the photometer to read the glucose concentration. Without delay after the cuvette sampling, capillary blood was also taken into a 50-μL microcup and transferred at once into the 200-μL solution of internal standard. The 50-μL microcup was washed five times with the [\textsuperscript{13}C\textsubscript{6}]glucose solution, and the sample was thoroughly mixed. These tubes (containing the mixture of blood and internal standard
solution) were brought to the laboratory and analyzed by ID GC-MS as described above.

For accuracy control in the comparison study, we measured a control sample (Seronorm) with every batch of blood samples analyzed in parallel by both the HemoCue method and the ID GC-MS method. The Seronorm was analyzed by both methods. The accuracy of the HemoCue analyzer was also checked for every sample batch by testing of the control cuvette.

**Results and Discussion**

**Analytical procedure and glycolysis study.** The influence of glycolysis when measuring glucose in whole blood is crucial. Depending on the hematocrit, glycolysis reduces the glucose concentration by 7–23% in 1 h and by 25–50% in 4 h after blood collection [14]. This emphasizes that the effect of glycolysis must be eliminated if one is to get correct results when measuring glucose concentration in whole blood. Glycolytic inhibitors such as sodium fluoride reduce the effect of glycolysis only a very little in the first 1–2 h after blood collection [14, 15]. In the case of bedside glucose monitors, which analyze capillary blood immediately after collection, the effect of glycolysis is eliminated. In the method presented here, glycolysis is effectively inhibited when 50 μL of capillary blood is taken into 200 μL of internal standard solution—as confirmed in a test where glucose was measured in three samples of blood taken into EDTA K$_2$-containing tubes (Table 1). We saw no decrease at all in glucose concentration for 240 min in blood samples so collected; a stable glucose concentration was reached within a few minutes. The standard protocol we propose calls for the samples to be left for 1–2 h after collection of blood to ensure equilibrium between blood glucose and [13C$_6$]glucose before the addition of methanol.

When performing isotope dilution analysis, one must make sure that equilibrium is reached between the internal standard and the substance to be measured. This is especially important for whole blood, which is not homogeneous and differs in glucose concentration between plasma and the erythrocytes. In the method we present, problems with this concentration gradient are overcome by adding 50 μL of blood to 200 μL of an aqueous solution of the internal standard and mixing thoroughly. The erythrocytes are lysed in this solution and the intracellular glucose is released. We found that deproteinization could be performed 1–2 h after mixing the blood and the internal standard solution because full equilibrium between glucose and [13C$_6$]glucose was achieved in <1 h.

In the glycolysis test, all of the blood samples had normal hematocrit values. Sidebottom et al. [14] showed that, in a blood sample with a hematocrit of 0.43, the glucose concentration decreased ~25% in 4 h; in a sample with a hematocrit of 0.75, the glucose concentration decreased ~50%. In this test, where we mixed 50 μL of whole blood with 200 μL of internal standard solution, no decrease in glucose concentration was apparent after 4 h. In view of the dramatic decrease of glucose concentration in nontreated blood samples [14], we conclude that the inhibition of the glycolysis in the method described is independent of the hematocrit of the blood.

**Precision and accuracy.** Within-run imprecision was checked by multiple analyses of glucose in whole blood, Seronorm, Pathonorm L, and Pathonorm H (Table 2). To check the between-run imprecision, we analyzed Seronorm as a control in different series of blood samples on 36 different days over 10 months. Calculations from these analyses showed that the between-run CV was 1.44% (n = 36). Pathonorm L and Pathonorm H were analyzed similarly, on 19 and 21 different days, respectively. The method showed good precision in all materials and at the three concentration ranges tested.

To check the contribution of the pipetting steps to the imprecision of the method, we weighed 20 empty microcaps and then reweighed them after filling each with 50 μL of water at 22 °C, calculating from the density of water the volumes delivered by the microcaps. The imprecision (CV) of this pipetting step was 0.44%. The exact volume of this step in the analysis procedure is not crucial because we used the same type of microcap to deliver the standard solutions. The imprecision (CV) of pipetting the internal standard was 0.11%, determined by weighing 20 times

### Table 1. Effect of glycolysis on glucose in three blood samples in internal standard solutions.

<table>
<thead>
<tr>
<th>Time after mixing, min</th>
<th>Glucose concn., mmol/L</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.37</td>
<td>3.05</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4.32</td>
<td>3.05</td>
<td>3.35</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>4.39</td>
<td>3.06</td>
<td>3.36</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>4.39</td>
<td>3.04</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>4.41</td>
<td>3.06</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>4.41</td>
<td>2.96</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>4.36</td>
<td>3.06</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.38</td>
<td>3.04</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.034</td>
<td>0.038</td>
<td>0.030</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Within-run (Wr) and between-run (Br) imprecision with whole blood and control sera.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Glucose, mmol/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wr</td>
<td>Br</td>
</tr>
<tr>
<td>Whole blood</td>
<td>2.28</td>
<td>2.25</td>
</tr>
<tr>
<td>Whole blood</td>
<td>5.31</td>
<td>5.00</td>
</tr>
<tr>
<td>Whole blood</td>
<td>18.61</td>
<td>ND</td>
</tr>
<tr>
<td>Pathonorm L</td>
<td>2.26</td>
<td>2.65</td>
</tr>
<tr>
<td>Seronorm*</td>
<td>5.28</td>
<td>5.00</td>
</tr>
<tr>
<td>Pathonorm H</td>
<td>11.91</td>
<td>12.75</td>
</tr>
</tbody>
</table>

*a Lot no. 503418 for within-run; lot no. 182 for between-run.

*b Mean of single analysis.

*c No. of runs is given in parentheses.

ND, not determined.
200 μL of water at 22 °C as delivered by the Finnpipette used for the internal standard solutions.

The largest contribution to the total imprecision of the method was from sample delivery with the microcap. This sampling technique is nevertheless unavoidable if capillary blood is to be taken. In all concentration ranges (low, normal, and high), the between-run imprecision is low, with CVs of 1.79%, 1.44%, and 1.57%, respectively, and should be compared with the CV limit of 2.0% for a glucose Reference Method proposed in the German program for Quality Assurance in Medical Laboratories [16].

The accuracy of the method was assessed by analysis of SRM 909b Level I. The accuracy test was performed with standard solutions of glucose prepared from both Mallinckrodt and SRM 907a (SRM 907a was not available at the start of this study, hence the use of the Mallinckrodt glucose). The mean ± SD concentrations of glucose in SRM 909b Level I were 5.41 ± 0.093 mmol/L (n = 10) with Mallinckrodt standard solutions and 5.38 ± 0.047 mmol/L (n = 10) with SRM 917a standard solutions. The results are well within the concentration range certified by NIST for glucose in reconstituted SRM 909b Level I (i.e., 5.40 ± 0.28 mmol/L).

**Linearity.** The mass spectra of the aldononitrile pentaacetate derivatives of glucose and [13C6]glucose are shown in Fig. 1. The ratio of m/z (314.0 + 242.1) for glucose to m/z (319.0 + 246.1) for [13C6]glucose was used for linear regression fit. To study the accuracy of the HemoCue blood glucose analyzer, we used six standard solutions with glucose concentrations of 1.0–20.0 mmol/L, a range within which the standard curve was found to be linear. The linear regression data for a representative standard curve had a slope of 0.228 and an intercept of 0.026, with a correlation coefficient of 1.000 and dispersion around the regression line (S_yx) of 0.0178 mmol/L.

**Specificity.** The specificity of the method was checked by analyzing blood from an EDTA K3-containing tube that had been gently mixed for a little more than 3 days. The blood was analyzed by the routine protocol described above, and a peak eluting at the same place as glucose, i.e., at 11.41 min, was hardly detectable (Fig. 2B). When the analysis was carried out without the addition of [13C6]glucose, no peak at all could be seen at the elution position of glucose (Fig. 2A). The small glucose peak seen in Fig. 2B, which probably originates from trace amounts of glucose in the [13C6]glucose batch used, was estimated as 0.2% of the amount of [13C6]glucose added. The peak corresponds to a glucose concentration of <0.01 mmol/L and its contribution to quantification of glucose in blood can be neglected. We conclude that the method is highly specific for analyzing glucose in whole blood.
Method comparison. In the accuracy study of the HemoCue blood glucose analyzer, capillary blood was taken from 67 patients with diabetes mellitus and 62 nondiabetic individuals. Samples were taken on two different occasions from three of the diabetic patients and from eight of the nondiabetic individuals. Single measurements were performed on all samples by both methods. Collecting single capillary blood samples for assay by both methods minimized any eventual alterations of blood glucose concentration during sampling. The two methods performed well enough to justify single measurements with the ID GC-MS and HemoCue method during the method comparison.

In all, 140 blood samples were measured in the comparison study: an average of 4–5 samples per day on 32 days. Moreover, in each batch of samples, we assayed Seronorm (lot no. 182) by both methods to check the accuracy and precision of the methods. At the same time, we also used the control cuvette to control the precision of the HemoCue instrument. The mean glucose concentration of Seronorm measured with HemoCue was 6.33 mmol/L (CV = 2.0%, n = 32). The HemoCue blood glucose analyzer is dedicated for measurements of glucose in whole blood and does not give correct concentration values in serum. The stability of the manufacturer’s calibration of the instrument was assured by repeatedly measuring the control cuvette. The result for this cuvette was 15.3 mmol/L at every measurement (n = 32) during the comparison study—the same as the target value for the instrument used.

Measurements of Seronorm by the ID GC-MS method during the comparison study are shown in Table 2. The mean concentration of glucose measured in Seronorm was 5.00 mmol/L (CV = 1.44%, n = 36).

For measurements of the 140 blood samples, the results from the HemoCue instrument were compared with those of the ID GC-MS method by a bias plot (Fig. 3) and linear regression analysis. The bias plot showed very good accuracy for the HemoCue instrument, especially for concentrations <10 mmol/L, where the mean HemoCue deviation from the ID GC-MS results was 0.04 mmol/L (or 0.9%), the maximal deviation being 0.95 mmol/L (9.8%). In the glucose range of 10–22.2 mmol/L, a positive bias was seen: mean 0.55 mmol/L (3.6%). The maximal deviation in this concentration range was 2.27 mmol/L (13.0%). The mean deviation over the whole concentration range was 0.24 mmol/L (2.0%). Note that the results from all samples taken in the method comparison are included in the bias plot and in the regression analysis; no outliers have been excluded. The only samples not included were those from six diabetic patients, who gave a result of HHH on the HemoCue analyzer, indicating that the glucose concentration exceeded the recommended measuring limit of the instrument, 22.2 mmol/L.

Regression analysis between the HemoCue instrument and the ID GC-MS method showed a slope of 1.051 (95% confidence interval: −0.016 to −0.428), and r = 0.994. Dispersion around the regression line (Sy|x) was 0.59 mmol/L. The HemoCue instrument performed well, especially in the concentration range <10 mmol/L. The positive bias found at concentrations >10 mmol/L have small clinical significance. In any case, the accuracy of the HemoCue glucose analyzer is well within the acceptable deviation limit set by an American Diabetes Association consensus conference [17]. The recommendation from this conference is that assaying with glucose monitoring instruments should give values that deviate <15% from those of a Reference Method. Of all 140 results from the HemoCue instrument, 69% were within 5% of the ID GC-MS method, 91% were within 10%, and all results were within 15% (actually, within 14.3%) of the ID GC-MS method. The need for accuracy of blood glucose monitoring devices is obvious from the comparison study by Havlin et al. [18], who compared the results of six different glucose meters with those of a Beckman glucose...
analyzer (comparison method) for a total of 496 blood samples. Of the results from all six instruments, 23% to 59% deviated by >15% from the comparison method.

In conclusion, the method described is to our knowledge the first ID GC-MS technique adopted for analysis of glucose in capillary whole blood. We used it to assess the accuracy of a bedside whole-blood glucose analyzer. To be able to use the ID GC-MS method as a Reference Method for bedside glucose determination, we adjusted the analysis procedure to better fit the clinical circumstances. The ID GC-MS protocol is easy to follow, and both precision and accuracy are good enough to regard the method as a Reference Method for whole-blood glucose determinations.

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References