upper range of linearity is ~2000 mg/L. Thus this method may be performed with meconium samples containing HbF as little as 10% of total Hb by weight, for which the original color of the specimen may be brownish-green. Samples with relatively high Hb content may need to be diluted. To obtain the proper dilution, use a freshly made hemolysate containing 20 μL of blood in 5 mL of water for comparison.

There are no commercial quality-control samples available for assay of these specimens. However, control specimens can be easily prepared by lysing fresh blood cells in deionized water to generate a Hb solution of ~10 mg/L. A positive blood specimen containing HbF should also be used. These solutions can be aliquoted into 1.5-mL fractions and, stored frozen or refrigerated, should be stable for at least 6 months. Stored at 4°C, HbF decreases by ~10% after 1 year.

An HbF content >50% probably indicates that the blood is fetal in origin. Blood with HbF <10% definitely comes from the mother. Obviously, this procedure cannot distinguish fetal blood from certain hemoglobinopathies such as hereditary persistent HbF.

An average error for this method is ~10% (Table 2). An experimental error of this magnitude will not affect the deduction of source of blood in the meconium. Consequently, the spectrophotometric modification of the Apt test can unambiguously distinguish the origin of blood in a newborn's discharge.

References

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HemoCue β-Glucose Photometer Evaluated for Use in a Neonatal Intensive Care Unit

Elizabeth Vadasdi1 and Ellis Jacobs1,2,3

We evaluated the HemoCue β-Glucose Photometer system for use in our neonatal intensive care unit by assaying 178 heparinized whole-blood samples obtained by heel stick. The required sample size is 5 μL. Plasma glucose was analyzed by a glucose oxidase/oxygen electrode methodology. Across the glucose range of 1.28–21.87 mmol/L, the regression slope was 0.976 (r = 0.976, Sxy = 0.475). For samples with hematocrit ≤0.30, the regression slope was 0.981 (r = 0.950, Sxy = 0.415); for hematocrit of 0.31–0.49, the regression slope was 0.984 (r = 0.972, Sxy = 0.508); and for hematocrit ≥0.50, the regression slope was 0.959 (r = 0.988, Sxy = 0.394).

Human whole blood, bovine whole blood, and bovine serum-based quality-control materials were studied. Except for assays of the low-concentration human control material, the total CV was <3.5%. The accuracy and precision of the HemoCue system were comparable with those of conventional laboratory instrumentation.

Indexing Terms: reflectance photometry • point-of-care testing • hematocrit • critical care medicine • pediatric chemistry

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For the rapid assessment of glucose homeostasis, reagent strips impregnated with glucose oxidase have been available since the 1960s, and portable reflectance meters based on this principle have been marketed since 1970. By the 1980s, bedside capillary glucose monitoring had become a standard of practice even though several authors expressed concerns with the accuracy of the measurement (1–3). New and modified solid-phase reagent strips/meter systems were developed for utilization with the latest reflectance technology (e.g., Glucometer, Ames; One Touch, Lifescan; Accu Chek II, Boehringer Mannheim Diagnostics) and electrochemical technology (Satellite G, Medisense) (4–6). All these developments improved the accuracy of measurement, but none fully eliminated the effects of extreme hematocrit values on glucose determination in whole-blood samples.

In clinical practice, dry-reagent strip methods are widely used to screen for abnormal glucose concentration, most frequently for identifying neonatal hypoglycemia (7, 8). Several reports have emphasized (7–9) that, although dry-reagent strip technology is useful for screening glucose in neonates, it cannot be relied upon for monitoring glucose homeostasis. Thus, before any therapeutic intervention, confirmation by a conventional laboratory method is necessary.

In our previous investigation of the influence of he-
matocrit on glucose measurement in whole blood (10),
two of the systems we studied were dry-reagent strip/
reflectance meter systems: One Touch (LifeScan, Milpi-
tas, CA) and Accu Chek II (Boehringer Mannheim
Diagnostics, Indianapolis, IN). Hematocrits >0.65 sig-
nificantly affected the precision of both systems.
Because the normal range for hematocrit in newborn in-
fants is 0.55–0.65, neither system is applicable, as
indicated by previous authors, for accurate assessment
of glucose homeostasis in neonates. To eliminate the
effect of extreme hematocrit values on the accuracy of
glucose analysis, one may assay either serum or plasma
(11). However, use of these samples would necessitate a
centrifugation step, which would make the system cumber-
some to use at the bedside.

Ultimately, a system for quantifying whole-blood glu-
cose that is not significantly affected by hematocrit is
the ideal solution. The HemoCue β-Glucose Photometer
system (HemoCue AB, Angelholm, Sweden) reduces the
effect of hematocrit on the analysis by hemolyzing the
erthrocytes. This whole-blood glucose testing system
distributes disposable 5-μL cuvettes and a photometer.
The cuvette contains freeze-dried reagents deposited
uniformly in the cavity. The sample, which is drawn
into the cavity by capillary force, mixes with the re-
agents spontaneously. The erythrocytes are hemolyzed
by saponin, releasing the intracellular glucose. The α-D-
glucose content of the specimen is transformed to β-D-
glucose by the enzyme mutarotase (EC 5.1.3.3). Glucose
dehydrogenase (EC 1.1.1.47) catalyzes the oxidation of
β-D-glucose to form NADH. Via the action of diaphorase
(1.8.1.4), the NADH produced reduces methylthiazo-
lyldiphenyl tetrAZolium to a colored formazan dye.
When the chemical end point is reached, the formazan is
quantified photometrically. To eliminate the effect of
 turidity, the photometer uses dual-wavelength mea-
surement, at 660 and 840 nm.

The purpose of this study was to ascertain the viabil-
ity of HemoCue β-Glucose photometer for use in a neo-
natal intensive-care unit. Only neonatal specimens
were used in this study. Results were correlated to glu-
cose oxidase analysis of plasma specimens. Alternative
quality-control materials were studied.

Materials and Methods

Specimens. Whole-blood specimens anticogulated
with sodium heparin were selected from random from
those routinely received in the stat laboratory for blood
gas analysis. These specimens were obtained by heel
stick from premature or critically ill infants, <12
months old, in our neonatal intensive care unit. After
their arrival in the laboratory at room temperature, the
samples were immediately assayed with the HemoCue
β-Glucose system. Plasma was obtained by centrifuging
an aliquot for 2 min at 2540 × g in a Microfuge 12
( Beckman Instruments, Brea, CA) with a 13.2 fixed-
gle rotor. The plasma was analyzed for glucose with a
Beckman Glucose II Analyzer. All testing was done
according to manufacturer’s recommended procedures.
Microhematocrit was determined by centrifugation for
3.5 min at 15 225 × g in a Microfuge 12 with a micro-
ematocrit rotor. All specimens were analyzed in duplicate,
and testing was completed within 10 min of receipt.

Quality control. For quality control, three different mat-
erials at two concentrations each were used: a stabili-
zation human whole-blood matrix control, Meter Trax (He-
tronix, Benicia, CA), which requires storage at 2–8 °C; a
stabilized bovine whole-blood matrix control, Sugar-
Chek (Streck Labs., Omaha, NE), which was stored at
room temperature; and a bovine serum matrix control,
Test Point (Technicon Instruments, Tarrytown, NY),
which was kept at 2–8 °C before and after reconstitution.

Statistics. The National Committee for Clinical Lab-
oratory Standards (NCCLS) EP5 protocol for the assess-
ment of imprecision of an analytical system was utilized
(12). Paired Student’s t-test and least-squares linear regre-
sion analysis were used for the methodology com-
parison and to assess the influence of hematocrit.

Results

The within-run imprecision (CV) of the HemoCue sys-
istem was ascertained with the three types of quality-
control materials Table 1. The human whole-blood
control generated significant imprecision, CV 5.1%,
at the lower concentration. Both bovine controls, i.e.,
whole bovine serum, generated acceptable precision
(CVs 1.5–3.1%). However, after reconstitution, the
serum control was stable for only 8 days at 4–8 °C,
whereas the Sugar-Chek solution was stable for 30 days
after opening at room temperature. Therefore, we used
the Sugar-Chek material for the remainder of the study.

The effect of storage temperature on the microcuv-
ettes was measured for 30 days. Microcuvettes from
the same lot number were kept between 2 and 8 °C, as
recommended by the manufacturer; a duplicate was
kept at room temperature. Assay imprecision for both
types of cuvettes was ascertained with use of the Sugar-
Chek material and the NCCLS EP5 protocol (Table 2).
Table 3. Influence of Hematocrit on Accuracy of HemoCue \(\beta\)-Glucose Photometer and Correlation with Enzymatic Assay

<table>
<thead>
<tr>
<th>Hematocrit range</th>
<th>n</th>
<th>Mean (SD), mmol/L</th>
<th>Regression data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.187–0.77</td>
<td>178</td>
<td>5.17 (2.16)</td>
<td>Slope 0.9760, y-intercept 0.1486, (r^2) 0.976</td>
</tr>
<tr>
<td>&lt;0.31</td>
<td>23</td>
<td>5.55 (1.29)</td>
<td>Slope 0.9807, y-intercept 0.0193, (r^2) 0.950</td>
</tr>
<tr>
<td>0.31–0.49</td>
<td>115</td>
<td>5.17 (2.15)</td>
<td>Slope 0.9835, y-intercept 0.1205, (r^2) 0.972</td>
</tr>
<tr>
<td>&gt;0.49</td>
<td>40</td>
<td>4.84 (2.54)</td>
<td>Slope 0.9586, y-intercept 0.2740, (r^2) 0.988</td>
</tr>
</tbody>
</table>

The difference was statistically significant (\(P < 0.005\)) between the low control results obtained with the cuvettes that had been stored refrigerated vs those stored at room temperature, the later giving the higher results. Additionally there was greater imprecision of the assay at both concentrations when refrigerated cuvettes were used. Storage of cuvettes at higher temperatures reduced both the intrarun and total imprecision of the assay to <4%.

On 178 paired whole-blood and plasma samples obtained by heel stick, glucose values were determined with both the HemoCue system and the glucose oxidase/oxygen electrode methodology (Beckman Glucose II Analyzer). Paired Student’s t-test showed no statistically significant difference between the mean values (Table 3) of the two methods over a hematocrit range of 0.185–0.72 and a glucose range of 1.28–21.87 mmol/L. Furthermore, linear regression analysis demonstrated excellent correlation of the two methodologies, with a slope of 0.976 (\(r = 0.976\)).

To ascertain the influence of hematocrit on the whole-blood glucose measurements with the HemoCue system, we separated the data into three subgroups based on the hematocrit (Table 3). There was no difference in either the accuracy or the correlation of the HemoCue system when broken down by hematocrit range (Figure 1). Of particular importance was the fact that there was no statistically significant difference between the values determined for specimens with hematocrits >0.49.

Discussion

The purpose of this study was to determine whether the HemoCue \(\beta\)-Glucose Photometer is accurate and reliable for monitoring capillary blood concentrations of glucose in critically ill newborns. In neonates, the normal hematocrit values of capillary blood may be as high as 0.629 (±0.032) (13), and these high values are often accompanied by hypoglycemia. This combination is outside the reliable performance range of the currently used dry-reagent strip system and thus necessitates the use of conventional laboratory methodology for monitoring glucose homeostasis.

Glucose analysis in whole-blood from neonates is fraught with pitfalls. The presence of extreme hematocrit values could itself give misleading results (14–16)—a factor relevant mainly in small-for-date neonates with polycythemia, in whom a falsely low value may be cause for an unnecessary intravenous infusion of glucose. About 4% of the newborn population has polycythemia, with increased blood viscosity and hematocrit >0.65 (17). Additionally, about 1% of newborns has hyperviscosity caused by factors other than high hematocrits. The primary contributing factors to whole-blood viscosity are the number, size, and shape of erythrocytes and concentrations of plasma proteins (18). In the high hematocrit range, deformability of erythrocytes causes increased viscosity; as the hematocrit rises to >0.65, the viscosity increases progressively (19). Furthermore, Grosa et al. (20) found the deformability of preterm infants’ cells to be less than that of adult cells, resulting in an increased viscosity in the blood of infants. Because the dry-reagent strip systems, whether based on reflec-

![Fig. 1. Influence of hematocrit on correlation of HemoCue whole-blood glucose values to plasma values. All testing was performed as described in the text.](image-url)
tance meters or electrochemical detection systems, depend on a timed diffusion of glucose from the plasma into the test pad, an increase in sample viscosity will slow the diffusion and result in depressed values.

The results of the current study indicate that the analytical performance of the HemoCue system for testing whole-blood glucose in critically ill neonates closely approximates the values obtained with conventional laboratory instruments. There was no statistically significant difference in the results obtained by both methods, and the correlation was excellent, yielding slope, r, y-intercept, and \( S_{xy} \) values of 0.976, 0.976, 0.150, and 0.475, respectively. Moreover, there was no significant difference in the correlation studies when the data were separated into low, normal, and high hematocrit ranges. The influence of erythrocyte concentration, i.e., hematocrit, on the automated whole-blood glucose analysis is decreased significantly by hemolyzing the sample before initiating the chemical reaction. There is no significant change in the plasma glucose concentration upon hemolysis because the concentration of glucose in the water phase of both erythrocytes and plasma is identical (21). Furthermore, because the size (mean corpuscular volume) of neonatal erythrocytes is ~30% greater than that of adult erythrocytes (22), upon hemolysis, the dilutional effect of membrane proteins is less with neonatal than with adult specimens.

Data obtained with different quality-control materials indicate that the bovine serum chemistry control used in routine clinical laboratories is also a suitable control material for the HemoCue system. However, after reconstitution, the control is stable for only 8 days and has to be refrigerated, whereas the Sugar-Chek controls (stabilized bovine whole blood) were stable for 30 days after opening and are routinely stored at room temperature. The Meter Trak stabilized human whole-blood controls were also acceptable. However, the requirement of constant storage at 2–8 °C makes this material a less viable alternative in clinical practice.

The storage temperature of the microcuvettes affected the analytical performance of the HemoCue system. Storage at higher temperature significantly increased (P <0.005) the measured mean glucose value for the low-concentration control solution from 7.62 to 8.06 mmol/L. Additionally, there was an increase in precision when the nonrefrigerated rather than the refrigerated microcuvettes were used. This is probably because, according to the manufacturer’s procedure, the cuvettes are used immediately after removal from the storage container, so that the contents of the refrigerated cuvettes do not have sufficient time to temperature stabilize. This, in conjunction with fluctuations in room temperatures, probably causes a significant variation in the final reaction temperature. Thus, implementation of the HemoCue \( \beta \)-Glucose Photometer system as a point-of-care device requires careful consideration of the effect of temperature on the accuracy.

In conclusion, whole-blood glucose analysis with the HemoCue \( \beta \)-Glucose Photometer system is simple, accurate, and precise. Accuracy and precision comparable with those of “wet” chemistry methods were achieved by a system with minimal maintenance and a sample size of 5 μL. Results were not affected by extremes in sample hematocrits.

We thank HemoCue AB for supplying the materials used in this study.

References