The "Eyetone" Blood Glucose Reflectance Colorimeter Evaluated for in Vitro and in Vivo Accuracy and Clinical Efficacy

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We evaluated the performance of a blood glucose reflectance colorimeter ("Eyetone," Ames Co.) for accuracy and precision with use of "Dextrostix" (Ames Co.) glucose oxidase reagent strips for blood samples with known and unknown concentrations of glucose covering the usual range of neonatal blood glucose (200–800 mg/L). The meter was calibrated and tested by research nurses and one clinical chemist. Five unknowns were tested for accuracy and precision (56–92 determinations per unknown) and compared with Beckman Astra values (plasma and calculated whole blood). Eyetone/Dextrostix values differed (gave lower values) from the calculated whole-blood values only at concentrations <300 mg/L. On 258 clinical specimens from newborn infants, Eyetone/Dextrostix values were not different from calculated whole-blood values (p < 0.05, r = 0.80). Operator training to develop a consistent procedure was the most critical factor in achieving accurate and precise results.

Additional Keyphrases: pediatric chemistry · diabetes · glucose oxidase reagent strips

Glucose oxidase reagent strips (e.g., Dextrostix) have been adopted in many nurseries for routine screening of blood glucose concentration in babies. Previous evaluations of the accuracy of Dextrostix glucose determinations (estimated by visual color comparison) have shown wide ranges of values, especially in the low range of glucose concentration (e.g., <500 mg/L). For example, Frantz et al. (1) reported a standard error of Dextrostix values of ±300 mg/L for any given true glucose value. Such inaccuracy may preclude the distinction of "hypoglycemic" glucose values from "normoglycemic" glucose values, limiting diagnostic accuracy as well as the determination of the response to therapy.

Accuracy has been improved with electronic reflectance colorimeters, such as the Eyetone, which eliminate inevitable observer variations in making visual color comparisons. Several studies of glucose reflectance colorimeters have reported standard deviations for Dextrostix values ranging from 90 to 220 mg/L for any given true glucose value over a glucose concentration range of 0 to 4000 mg/L (2–4). These levels of accuracy are far from ideal for many clinical situations.

We undertook the present study to try to optimize the accuracy of Eyetone/Dextrostix glucose concentration measurements and to test the sensitivity of the Eyetone for detecting a change in glucose concentration in the "hypoglycemic" range (0 to 500 mg/L of blood) of newborn babies.

Materials and Methods

Five research nurses (registered nurses, trained in intensive care neonatal nursing) and one clinical chemist were trained to use the Eyetone instrument.

For the training period, known amounts of glucose were added to glycolyzed adult whole blood, produced by incubating blood at 37 °C until the glucose concentration was zero. Each member of the group assayed each sample until a within-run performance accuracy of <50 mg/L was achieved for samples in the blood glucose concentration range of 150–600 mg/L. Newly reconstituted samples were assayed weekly throughout the eight-month study period.

Each nurse performed the electronic calibration and standardization of the Eyetone before each run (both for in vitro and for clinical specimens) according to the routine operator's instructions. The Eyetone was specifically standardized for low glucose values, with a 400 mg/L standard solution.

Instrument precision and accuracy were assessed by parallel testing of blood at five known glucose concentrations (adult whole blood glycolyzed to 0 mg/L by incubation at 37 °C and reconstituted with known amounts of glucose) with Eyetone/Dextrostix on whole blood and a Beckman Astra analyzer (Beckman Instruments, Fullerton, CA 92634) on plasma. Because the Eyetone was calibrated to read glucose concentrations in whole blood, Astra plasma values were converted to whole blood according to the equation: blood glucose = plasma glucose [1.0 – (2.4 × 10⁻³ × hematocrit, %)] (4).

Similarly, skin-puncture (heel-stick) blood samples from newborn infants were assayed for glucose concentration by both the Eyetone/Dextrostix on whole blood and by the Beckman Astra on plasma. This part of the study was approved by the Human Subjects Committee of the University of Colorado Health Sciences Center.

Finally, Eyetone glucose concentrations were used to adjust intravenous glucose infusion rates on two babies to test the instrument's accuracy in measuring a therapeutic response (change in glucose concentration) to an intravenous glucose infusion, and to adjust the glucose infusion rate to achieve a desired glucose concentration. In these two infants, the glucose infusion rate was adjusted to achieve a desired blood glucose concentration according to a modified "glucose clamp" technique (5). Fequent blood glucose values were measured with the Eyetone and a Yellow Springs glucose analyzer (Yellow Springs Instruments, Inc., Yellow Springs, OH 45387). Selected glucose values were entered into a programmed calculator, which determined a new glucose infusion rate if the desired glucose concentration had not been reached.

Results

Table 1 presents the performance summary for reconstituted standard specimens. The mean standard deviation was 73 mg/L for the Eyetone/Dextrostix whole-blood glucose values, 27 mg/L for Beckman Astra plasma values, and 23 mg/L for Beckman Astra estimated whole-blood values. The mean Eyetone/Dextrostix value for each test glucose concentration was significantly less than the corresponding mean Astra plasma value (p < 0.05), and was significantly less (p
Table 1. Glucose Determinations by Eyetone/Dextrostix and Beckman Astra Performance Summary for Reconstituted Specimens

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Eyetone/Dextrostix(^a)</th>
<th>SD</th>
<th>Mean Beckman Astra(^b)</th>
<th>SD</th>
<th>Mean Whole blood(^c)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>72</td>
<td>66</td>
<td>148</td>
<td>25</td>
<td>134</td>
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<tr>
<td>200</td>
<td>149</td>
<td>64</td>
<td>205</td>
<td>24</td>
<td>184</td>
<td>21</td>
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<tr>
<td>300</td>
<td>278</td>
<td>71</td>
<td>316</td>
<td>30</td>
<td>285</td>
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<tr>
<td>400</td>
<td>398</td>
<td>81</td>
<td>432</td>
<td>30</td>
<td>389</td>
<td>26</td>
</tr>
<tr>
<td>700</td>
<td>600</td>
<td>81</td>
<td>637</td>
<td>25</td>
<td>574</td>
<td>21</td>
</tr>
</tbody>
</table>

* From 22 or 23 assay runs with an average of four replicate assays per run. \(^b\) From 14 assay runs with an average of four replicate assays per run. \(^c\) Beckman plasma glucose results converted to whole-blood glucose results (see text).

< 0.05) than Astra calculated whole-blood values at 150 and 200 mg/L concentrations.

The most important factor determining instrument accuracy and precision was the development of an appropriate and consistent operator technique. Three aspects of technique were most critical: (a) the drop of blood had to cover the entire Dextrostix reaction area; (b) reaction time had to be 60 ± 2 s or longer; and (c) washing the blood off the reaction area had to be consistent to achieve good replicate precision and to maintain accuracy. The latter point was the main reason why each operator greatly improved accuracy by individually calibrating the instrument. Once a particular operator's technique was consistent, there was no change in accuracy or precision over the eight-month study period.

For a comparison of 258 samples in which glucose concentrations were less than 800 mg/L, paired Eyetone/Dextrostix (y) and Beckman Astra (x) estimated whole-blood glucose values correlated at \( r = 0.80 \) (\( y = 0.4 + 1.04x \)). For 72 comparisons in which both Eyetone and Beckman Astra blood glucose values were less than 500 mg/L, the Beckman Astra values (mean ± SD, 337 ± 100 mg/L) and the Eyetone values (347 ± 109 mg/L) were better correlated (\( r = 0.85 \)) and not significantly different (\( p < 0.01 \)).

Table 2 compares the mean and 90% range values for Eyetone/Dextrostix values with paired Astra estimated whole-blood values over six 100 mg/L ranges. The mean Eyetone/Dextrostix values were not significantly different from the mean Astra values in each range (\( p > 0.1 \)).

Figure 1 presents whole-blood (Astra) glucose concentration, Eyetone/Dextrostix glucose concentrations, and glucose infusion rates for one patient, an infant of a diabetic mother. This infant's hypoglycemia followed maternal hyperglycemia during labor (caused by an intravenous glucose bolus) and two large intravenous glucose boluses (933 mg/kg body weight) into the infant. A sustained variable glucose infusion rate was adjusted every 10 to 15 min according to Eyetone/Dextrostix skin-puncture blood glucose values, which were determined starting at 3.5 h after birth. The variable glucose infusion pump was adjusted according to a calculator program modified from the euglycemic glucose clamp equation of DeFronzo et al. (5). Increasing glucose concentrations were obtained within 20 min, and sustained normoglycemia (Eyetone/Dextrostix values >500 mg/L) was obtained within 60 min. The infant was asymptomatic throughout the duration (6 h) of observation. The plateau glucose concentration of 900 mg/L required an average glucose infusion of 12.7 mg · min\(^{-1}\) · kg\(^{-1}\).

Figure 2 presents data from a 960-g, 29-week-gestation pre-term infant with established hyperglycemia at a glucose infusion rate of only 3.6 mg · min\(^{-1}\) · kg\(^{-1}\). To achieve normal glucose values, the patient's physicians administered insulin at 1.04 milli-units · min\(^{-1}\) · kg\(^{-1}\) by constant infusion. Eyetone glucose values for arterial blood from an indwelling umbilical artery catheter were determined every 10 min and compared with simultaneous plasma glucose concentrations measured at the bedside with the YSI glucose analyzer (glucose concentration accuracy, ± 10 mg/L). The glucose infusion rate was adjusted by the modified glucose clamp program by using the YSI glucose analyzer values to achieve a glucose concentration of 800 mg/L. The plateau glucose value (839 ± 21 mg/L, mean ± SEM) at 135–250 min was sustained with 15.8 ± 1.2 mg · min\(^{-1}\) · kg\(^{-1}\) of glucose infused. The dotted line represents the glucose infusion rates that would have been calculated if the Eyetone glucose values had been entered.

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Table 2. Estimated 90% Confidence Intervals for Eyetone/Dextrostix at Specific Astra Glucose Concentration Values

<table>
<thead>
<tr>
<th>Astra glucose concn, mg/L(^a)</th>
<th>No. of specimens in range</th>
<th>Eyetone/Dextrostix whole-blood glucose concn, mg/L(^a)</th>
<th>Mean</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Astra ± 50 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>4</td>
<td>210, 150, 270</td>
<td></td>
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<td></td>
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<tr>
<td>300</td>
<td>16</td>
<td>310, 150, 470</td>
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<tr>
<td>400</td>
<td>31</td>
<td>420, 270, 570</td>
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<td>38</td>
<td>510, 350, 670</td>
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</tr>
<tr>
<td>600</td>
<td>46</td>
<td>650, 430, 870</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>700</td>
<td>78</td>
<td>730, 510, 950</td>
<td></td>
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</tr>
</tbody>
</table>

* Converted to whole-blood values (see text).
The blood glucose concentrations are achieved by using insulin at 1.04 milli-units \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) started at time zero.

The glucose infusion rate (solid line) was variably adjusted according to the YSI glucose analyzer values; the broken line represents the glucose infusion rate that would have been calculated (9) for each Eyetone/Dextrostix glucose value into the calculator (mean ± SEM, 14.0 ± 1.9 mg \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) from 135 to 250 min).

**Discussion**

The accuracy and precision of the Eyetone/Dextrostix achieved in the present study compare favorably with the clinical chemistry laboratory glucose analyzers. Although the standard deviation of the clinical sample comparison between the Eyetone/Dextrostix and Astra estimated whole-blood samples of 123 mg/L is large enough that a baby with true hypoglycemia (assuming the Beckman Astra values are the true values) might be missed by the Eyetone/Dextrostix glucose determination, this happened with only 10 out of 72 determinations in the clinical comparisons in which the Beckman Astra value was 500 mg/L or less.

We emphasize that the maximal accuracy obtained with the Eyetone/Dextrostix required operator training, continuing practice sessions, and separate calibration by each operator. These requirements should be considered when using Eyetone/Dextrostix determinations of blood glucose in a clinical setting in which accurate and sensitive glucose concentrations are needed quite rapidly, especially when different operators would be using the same instrument. However, once a particular operator develops instrument familiarity and a consistent performance technique, accuracy and precision are easily maintained by that operator.

The higher standard deviation of the Eyetone/Dextrostix in vitro assays (39 mg/L within run; 61 mg/L between runs) compared with the Astra in vitro standard deviations (6 mg/L within run; 22 mg/L between runs) defines a limit of accuracy for the Eyetone that is less than the clinical chemistry lab can offer. The accuracy of the Eyetone/Dextrostix glucose values must be considered carefully when such values are used in clinical situations in which hypoglycemia must be detected and a response to therapy guaranteed. The successful use of the Eyetone/Dextrostix values to both monitor and guide intravenous glucose therapy in the two cases described suggests that the available accuracy and short turnaround time should be useful for rapid, safe correction of hypoglycemia and maintenance of normal glucose concentrations. This point deserves further testing. Certainly, the accuracy and precision of Eyetone glucose concentrations are a vast improvement over visual Dextrostix glucose values.

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**References**