Immediate HbA_{1c} Results

Performance of new HbA_{1c} system in pediatric outpatient population

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OBJECTIVE — This study compared the performance of a new device that uses an IA to measure HbA_{1c} in 9 min with a 1-μl capillary blood sample with AC and CE methods in both nondiabetic and diabetic pediatric patients.

RESEARCH DESIGN AND METHODS — Two hundred seven pediatric subjects (103 nondiabetic, 104 with insulin-dependent diabetes mellitus) had HbA_{1c} measured with the IA method and compared with total GHb values determined by AC and HbA_1 by the CE method with the same whole-blood capillary aliquot. Glucose values were also obtained from the same blood samples.

RESULTS — Correlations and regression analyses show excellent correspondence between the three assays. The correlation between the AC and CE methods is 0.98 ($P < 0.001$) with a slope of 1.615 ± 0.0125 and intercept of 4.00 ± 0.20. The correlation between the IA and AC methods is 0.99 ($P < 0.001$) with a slope of 0.608 ± 0.007 and intercept of 1.326 ± 0.066. The correlation between the IA and CE methods is 0.97 ($P < 0.001$), with a slope of 0.983 ± 0.018 and intercept of 1.122 ± 0.153. The average difference and average percentage difference between methods were also significant ($P < 0.001$), reflecting the differences in GHb components measured. There was a significant correlation ($P < 0.001$) between each method and glucose values (IA $r = 0.72$, AC $r = 0.70$, CE $r = 0.73$). Within-run precision for IA ranged from 1.7 to 3.5% and between-run precision 2.7 to 4.1%.

CONCLUSIONS — Study results suggest that the IA method gives extremely accurate and reliable values over the clinical range of interest. The instrument is small, portable, easy to use, and provides information within 9 min for both physicians and patients.

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IA, IMMUNOASSAY; AC, AFFINITY CHROMATOGRAPHY; CE, CATION EXCHANGE; GHb, GLYCOHEMOGLOBIN; C.V., COEFFICIENT OF VARIATION.

Measurement of GHb is widely recommended for evaluating the glycemic control of patients with diabetes mellitus (1,2). Recent evidence suggests that regular measurements of GHb and discussion of results with patients lead to changes in diabetes treatment and improvement in metabolic control (3). However, skepticism about the clinical usefulness of GHb measurements may persist due in part to delays in obtaining the result. Unless ordered "stat", current methods do not allow the physician access to the result of the test during the clinic encounter with the patient.

The DCA 2000 HbA_{1c} System (Miles, Diagnostic Division, Elkhart, IN) enables the physician to obtain an HbA_{1c} result in 9 min with a 1-μl sample of capillary or venous whole blood. Our study was designed to evaluate the performance of the DCA 2000 compared with two commonly available laboratory GHb assays in both nondiabetic and diabetic pediatric patients and to establish a pediatric normal range for the IA method.

RESEARCH DESIGN AND METHODS

DCA 2000 system

The DCA 2000 uses an IA based on the inhibition of latex agglutination and a monoclonal antibody specific for an amino acid sequence on the HbA_{1c} molecule (4,5). Therefore, removal of labile GHb is unnecessary. The assay principle and chemical reactions required are depicted in Figs. 1 and 2, respectively.

The system consists of three parts: the DCA 2000 chemistry analyzer (a spectrophotometer; Fig. 3), which is programmed by a magnetic card containing assay and instrument parameters; a sampling device that holds a 1-μl capillary tube; and a unitized reagent cartridge that contains all reagents necessary for the measurement of HbA_{1c} (Fig. 4). Each cartridge can only be used for one test.

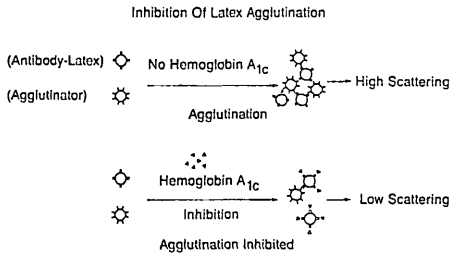

Figure 1—*Assay principle used in determination of HbA_{1c} concentrations. Slope of inhibitor and latex agglutination curve is sigmoidal. A 4-parameter curve fit is used to plot response vs. concentration for calculations of unknowns.*

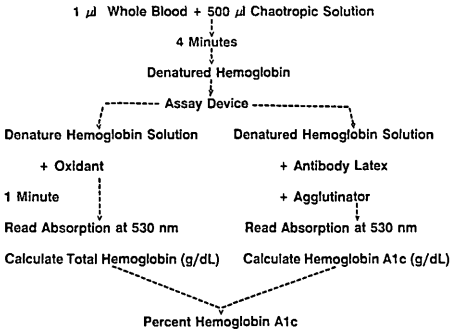

Figure 2—*Chemical reactions occurring within reagent cartridge. Ratio of HbA_{1c} to total Hb is calculated to determine percentage HbA_{1c}.*

Electronic calibration is performed by passing a bar-coded card past the instrument's reader. This card carries lot-specific calibration parameters and is required before the use of a particular reagent lot is begun. There are 12 calibrater points used to establish the calibration curve for two lots of reagents that are stored in the instrument's software. A high-performance liquid chromatography system (6) was used as the reference method for calibrating the system. No other instrument function checks are necessary. Reagents are stable over an 18-mo shelf life at 5°C. Quality control materials consist of lyophilized blood hemolysates.

To obtain a result, the operator collects a 1-µl sample of blood from a finger prick by capillary action in a sampling device, places it in a reagent cartridge, draws the cartridge through a bar-code reader on the instrument, places it into the instrument, reconstitutes the dried reagents by pulling a tab in the cartridge, and activates the test sequence by closing the reaction chamber door. The result, percentage HbA_{1c}, is displayed on a screen on the instrument 9 min later.

We studied 207 children and adolescents (103 nondiabetic [mean age 14.5 ± 3.5 yr] and 104 with IDDM [mean age 13.5 ± 4.3 yr, mean duration of diabetes 5.8 ± 4.0 yr]). Nondiabetic subjects were excluded if there was documented evidence of any disease, medication that could affect the test outcomes, or if their parents had diabetes. Because sex differences have not been demonstrated, no efforts were made to balance groups by sex.

Subjects with diabetes were solicited from the Diabetes Research and Training Center's pediatric diabetes clinical facility at Riley Hospital for Children. Individuals were excluded if there was documented evidence of any comorbidity or medication that could affect the test results. These studies received institutional review board approval, and informed consent was obtained before participation.

Subjects had their HbA_{1c} measured with the DCA 2000, which was compared with total GHb values determined on the same whole-blood capillary aliquot by AC (Glyc-Affin, Isolab, Akron, OH) and HbA_1 by CE (Quik-Sep, Isolab). Samples for AC and CE determinations were kept at 4°C for <1 wk before analysis.

In addition to HbA_{1c}, total GHb and HbA_1 glucose values were obtained for all subjects from the same blood samples. Glucose was determined on a 50-µl whole-blood capillary aliquot with the Hexokinase/G6P-DH with deproteinzation method (Boehringer Mannheim, Indianapolis, IN).

Random capillary blood samples were obtained from each subject's finger with a lancet device. Samples for all four

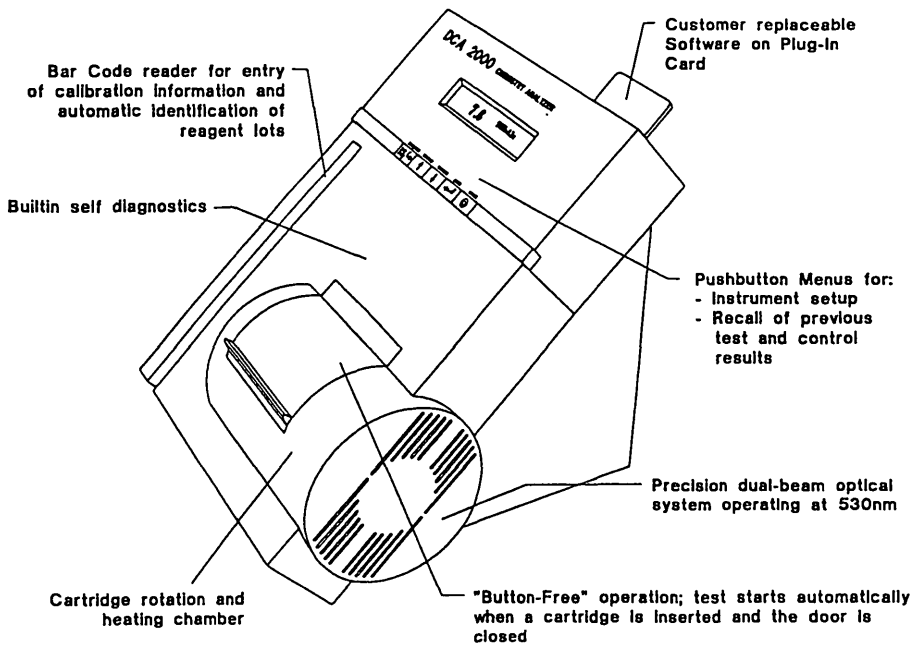

Figure 3—*DCA 2000 chemistry analyzer.*

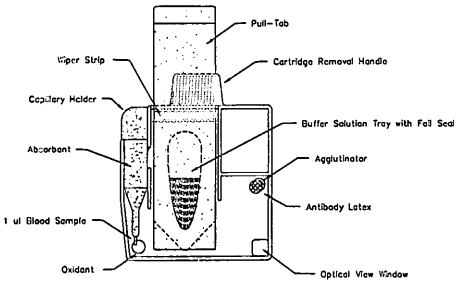

Figure 4—*DCA 2000 system blood sampling device and unitized reagent cartridge.*

measures were obtained from the same finger prick. The sample for the DCA 2000 was processed immediately as described above, and the results were recorded. The cartridge lot was systematically varied to achieve an even distribution between each of two lots. A quality control test was conducted both before and after each run for the first 7 runs and at every 15 tests thereafter. Three levels of quality control materials were used at alternating intervals. The quality controls were run in duplicate each time for determination of within-run precision.

The blood sample for the CE and AC assays was collected from the same finger prick in a 50-μl capillary tube and transferred to heparinized tubes that were kept refrigerated. Tests were performed in the Immunoassay Laboratory of the Indiana Univ. School of Medicine Diabetes Research and Training Center with kits and protocols supplied by the manufacturer. Separate lots of external controls and standards were used in each assay for quality control purposes (the same controls and standards cannot be used for both methods).

The Glyc-Affin assays were performed after minicolumns and reagents were permitted to equilibrate at room temperature, and columns were prepared according to manufacturer's instructions. Ten microliters of well-mixed capillary whole blood was added to 80 μl sample preparation reagent, vortexed, and permitted to sit at room temperature for 10 min, and 50 μl hemolysate was added to the top disc of the minicolumn. Intra- and interassay C.V.s of the AC method are <3%.

Because of the temperature dependence of the Quik-Sep method, reagents and columns were kept and run at 23°C in an environmental chamber. Twenty microliters of well-mixed whole-capillary blood was added to 80 μl labile fraction eliminator at room temperature. The hemolysate was vortexed and allowed to stand for 60 min with occasional remixing, and 50 μl hemolysate was added to the top of a disc of a prepared minicolumn in the environmental chamber. Intra- and interassay C.V.s of the AE method with an environmental chamber are 2%.

RESULTS— Table 1 shows the descriptive statistics for the nondiabetic and diabetic subjects respectively. The glucose values for both cohorts differed significantly (5.05 ± 0.93 and 13.69 ± 6.64 mM, respectively). Mean values for percentage HbA_{1c}, GHb, and HbA_1 differed significantly among the two groups, reflecting differences in GHb fractions measured by the three methods studied.

Correlations and regression among the three methods were also calculated. Figure 5 shows the relationship between the results obtained with the AC and CE methods for measuring GHb. The correlation is 0.98 ($P < 0.001$) with a slope of 1.615 ± 0.0125 and intercept of 4.00 ± 0.20. Outlier values were not investigated because sufficient sample volume was unavailable. However, the AC method is not susceptible to effects of abnormal Hbs. Although the CE method is sensitive to Hb effects, the use of a labile intermediate eliminator and an environmental chamber precludes effects of labile adducts and temperature. Figures 6 and 7 show the relationship between results obtained with the DCA 2000 and the Glyc-affin and Quik-sep methods, respectively. The correlation between the DCA 2000 and the Glyc-affin methods is 0.99 ($P < 0.001$) with a slope of 0.608 ± 0.007 and intercept of 1.326 ± 0.066. The correlation between the DCA 2000 and Quik-sep methods is 0.97 ($P < 0.001$) with a slope of 0.983 ± 0.018 and intercept of 1.122 ± 0.153.

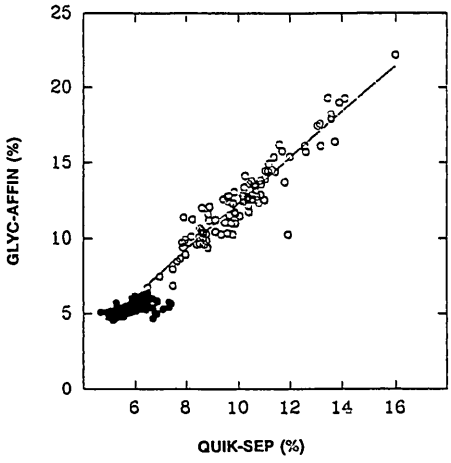

Figure 5—*HbA_1 measured by Glyc-Affin (total GHb) and Quik-sep (HbA_1) assay methods.*

Table 1—***Mean GHb and glucose for nondiabetic (N = 104) and diabetic (N = 103) subjects***

Assay	Nondiabetic	Diabetic
DCA 2000 (% HbA_{1c})	4.54 ± 0.30 (3.80–5.30)	8.96 ± 1.68 (5.10–14.00)
Glyc-affin (% GHb)	5.41 ± 0.38 (4.55–6.36)	12.48 ± 2.89 (6.30–22.14)
Quik-sep (% HbA_1)	5.92 ± 0.54 (4.68–7.39)	10.11 ± 1.79 (6.43–15.97)

Values are means ± SD with ranges in parentheses. DCA 2000 measures HbA_{1c} ≤14%; therefore, for values >14%, 14 was used in analysis.

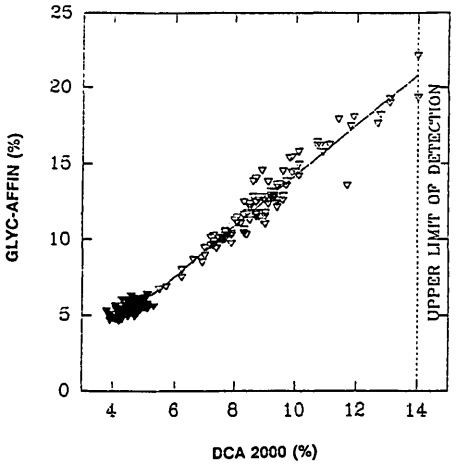

Figure 6—*HbA_{1c} and HbA_1 measured by DCA 2000 and Glyc-Affin methods.*

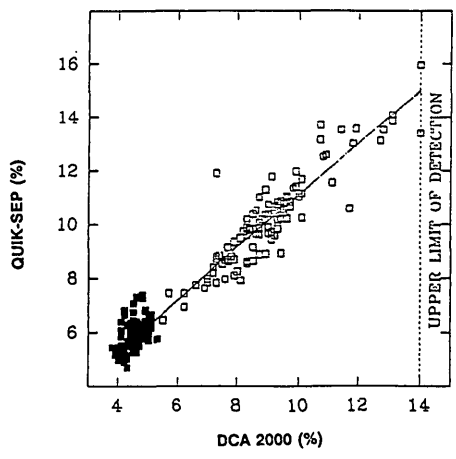

Figure 7—*HbA_{1c} and HbA_1 measured by DCA 2000 and Quik-sep methods.*

The correlations between each method and glucose values was calculated. As expected, HbA_1 and HbA_{1c} levels were significantly correlated with glucose levels. The strength of the correlation were $r = 0.705$ for Glyc-affin, $r = 0.731$ for Quik-sep, and $r = 0.723$ for DCA 2000 (all $P < 0.001$).

Finally, the precision of the DCA 2000 system is illustrated in Table 2. Within-run precision ranged from 1.7 to 3.5% on the three levels of control subjects. Between-run precision ranged from negligible to 2.3%, and overall precision ranged from 2.7 to 4.1%.

CONCLUSIONS— The use of GHb is an important tool in the management of individuals with diabetes mellitus. However, the clinical utility of this measure is compromised by limitations inherent in most assay methods. Measurement techniques often tend to be slow and labor intensive. Because of this, results are usually unavailable to the clinician at the time of consultation. Thus, the clinician cannot use the data in making treatment decisions at the clinic visit or providing prompt patient feedback. This is particularly problematic when treating patients who are difficult to contact by telephone or do not maintain regular clinic visits.

The results of our study suggest that the DCA 2000 HbA_{1c} system allows virtually immediate information for both physicians and patients. Our data also suggest that the system gives accurate and reliable values over the clinical range of interest. There was excellent correspondence between the three assays. We elected to make these comparisons with two commonly used assays for measuring GHb. Comparisons with the "gold standard" high-performance liquid chromatography method have demonstrated similar results ($r = 0.963$) (5). The assay is specific for HbA_{1c}, and the overall precision of the system is very good throughout the useful clinical range.

The technology and design of the DCA 2000 system offers several potential advantages over more conventional methods for measuring GHb. The assay is based on a specific monoclonal antibody. The stability of the assay cartridge system is electronic calibration and ease of use make the system particularly suitable for the physician's office. Moreover, the small size and portability of the instrument enables it to be used in the patient-examining room. This instrument enables the clinician to provide the patient with immediate feedback concerning the course of treatment. In addition, obtaining the blood sample by finger prick makes the system more acceptable for pediatric patients compared with methods that require venipuncture. We conclude that the DCA 2000 represents an important advance in the care of patients with diabetes mellitus.

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Table 2—*Statistical summary of DCA 2000 precision on 3 levels of HbA_{1c} control subjects*

Assays (N)	Control level	Target*	Mean (% HbA_{1c})	Within run SD	Within run % C.V.	Between run SD	Between run % C.V.	Overall SD	Overall % C.V.
26	A	5.2	4.87	0.17	3.5	0.11	2.3	0.20	4.1
26	B	7.99	7.48	0.26	3.2	Negligible	Negligible	0.25	3.2
26	C	12.0	11.58	0.20	1.7	0.24	2.1	0.31	2.7

Precision data was obtained before each of the 26 subject data collection sessions.
*Target values of control subjects were established by taking the unassayed control materials and making repetitive runs in the Miles Laboratories Quality Assurance and Research and Development laboratories.

Institute for Health Care (Indiana Univ. School of Medicine).

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