Alternate Site Testing for HbA_{1c} Using the Primus CLC330 GHb Analyzer

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OBJECTIVE — To determine whether the Primus high-pressure liquid chromatography (HPLC) is suited to alternate site testing (AST) for HbA_{1c} in a hospital diabetes outpatient clinic.

RESEARCH DESIGN AND METHODS — Patients were attending the clinic for routine management of their diabetes. A number of diabetic patients with uremia (n = 11) were also investigated. HbA_{1c} levels were measured in the outpatient setting by the Primus HPLC and in a more limited study the DCA-2000 instrument using the new 6-min assay cartridge. HbA_{1c} measurements were also performed with Pierce affinity minicolumns and a Bio-Rad Variant HPLC.

RESULTS — The Primus HPLC assay had low imprecision of 2.3, 1.6, and 1.0% for HbA_{1c} levels of 4.7, 7.3, and 11.1%, respectively, and was not prone to interference by carbamylated hemoglobin as found for the ion-exchange Variant HPLC method. Method comparison studies showed that the bias and proportional error between the Pierce affinity minicolumn procedure (standardized with respect to an external quality control program) and the Primus HPLC (Y) was −0.4 and 1.2% respectively (n = 32). Similarly the bias and proportional error between the Primus and DCA-2000 methods was 0.7 and −2.5%. The Primus was shown to give falsely elevated HbA_{1c} concentrations if the time between sequential injections was >28 min.

CONCLUSIONS — The Primus HPLC has a decided advantage over specialty AST instruments, like the DCA-2000, in not only meeting AST requirements but also allowing rapid automated batch processing of all laboratory HbA_{1c} samples.

The significance of GHb in the clinical management of patients with diabetes has been emphasized by the recent Diabetes Control and Complications Trial (DCCT) findings (1). Results for the latter trial indicate that maintenance of very tight blood glucose control will significantly reduce the long-term complications of diabetes, particularly retinopathy and nephropathy. Ideally GHb results would be available at the time of patient's consultations, reducing clinician workload in following up results and allowing immediate changes in therapy as indicated by the GHb level. Although a plethora of methods exist for the measurement of GHb (2), few are able to satisfy the practical requirements of a busy outpatient clinic. The most important factor in this type of setting is turnaround time from specimen collection to result reporting.

Our own past experience with alternate site testing (AST) for blood glucose with a YSI analyzer (Yellow Springs, OH) suggests turnaround times of ~5 min are necessary. In this study we report our findings for AST of HbA_{1c} within a hospital diabetic outpatients setting using the Primus CLC330 high-pressure liquid chromatography (HPLC).

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AST, alternate site testing; DCCT, Diabetes Control and Complications Trial; HPLC, high-pressure liquid chromatography.

RESEARCH DESIGN AND METHODS

Subjects and blood sample collection

The subjects were attending the diabetic outpatient clinic for management of their diabetes. Immediately upon arrival at the clinic, subjects have a 10-ml blood specimen collected by antecubital venipuncture into tubes containing lithium heparin. During the trials a small aliquot of this sample was used for the HbA_{1c} onsite measurement (Primus or DCA-2000) and the remainder was forwarded to the central pathology laboratory via a computerized pneumatic tube delivery system, principally for rapid measurement of blood glucose (YSI, 23AM glucose analyzer) and subsequent routine batch determination of HbA_{1c} (Variant HPLC). The use of a pneumatic tube delivery system for measurement of blood glucose was being tried as an alternative to the normal practice of performing these determinations onsite. A subsequent review concluded the latter system could not achieve the necessary turnaround times. Blood samples were also collected from 11 diabetic patient with elevated plasma urea concentrations (median 22.6 mmol/l, range 8–155 mmol/l; normal range 3.0–7.6 mmol/l).

HbA_{1c} methods

The standard laboratory procedure uses Pierce boronate affinity minicolumns (3) where the measured GHb value is converted to an HbA_{1c} equivalent using a regression equation derived against an ion-exchange HPLC method. The validity of this approach is verified by comparison of HbA_{1c} levels by our method versus the Bio-Rad Diamat HPLC (DCCT reference method) group mean in the SKZL External Quality Control Program (4); during a 2-year period (n = 52), the bias was 0.05% and the proportional error, 0% (5). The Primus CLC330 GHb Analyzer (Primus, Kansas City, MO) is an HPLC procedure employing a short boronate affinity resin column, maintained at 48°C, to allow rapid group separation of glycated and nonglycated hemoglobins; the actual GHb value is then converted to a respective HbA_{1c} equiv-

Table 1—*Imprecision of Primus HPLC assay for HbA_{1c}*

	n	Mean HbA_{1c}	Cv_{within}	$Cv_{between}$	CV_{total}
Level I	85	4.7	2.3	0.02	2.3
Level II	64	7.3	1.3	1.0	1.6
Level II	61	11.1	0.9	0.5	1.0

The number of different day batch assays for I, II, and III was 13, 12, and 12, respectively.

alent based on a predetermined algorithm (6,7). The Bio-Rad Variant HPLC (Bio-Rad, Melbourne, Australia) uses an ion-exchange resin to chromatographically separate the different hemoglobins and thus reports HbA_{1c} directly. The DCA-2000 (Bayer, Pymble, Australia) is a portable instrument which uses an immunoassay specific for the HbA_{1c} amino acid sequence; all reagents are held in a self-contained cartridge (8,9). The DCA-2000 was supplied with the new 6-min assay cartridges (previously 9-min). The Primus CLC330 GHb Analyzer and DCA-2000 were operated by the main laboratory's scientific staff.

Statistical methods

Equality of HbA_{1c} levels between different assays was compared by the Passing-Bablok regression procedure (5), which makes no special assumptions regarding the distribution of samples and measurement errors. Imprecision of the Primus HPLC method was calculated using an analysis of variance with unequal sample sizes. Differences between independent group means were assessed by the Mann-Whitney *U* test, while the Fisher *Z* test was used for matched samples.

RESULTS — Before AST, several aspects of the Primus instrument's operation were investigated. Potential errors due to carryover effects between sequential injections were evaluated using blood samples having a low (4.6–4.8%) and high (14.2–14.5%) concentration of HbA_{1c}. The precision of measurement was found for both high and low HbA_{1c} specimens. The high HbA_{1c} sample was then injected 5 times followed by 5 injections of the low HbA_{1c} sample; this sequence was repeated several times. In this way the maximum absolute error arising from possible carryover was estimated at <0.2%. The within- and between-day imprecision of the Primus method is summarized in Table 1. Comparison of the Primus (Y) and Pierce (X) methods showed a bias of −0.4 (95% CI: −0.7 to −0.1) and a proportional error of 1.2% (95% CI: −2.5 to 4.4%) (Fig. 1); the corresponding mean HbA_{1c} levels for the sample set (n = 32) were 8.1 ± 2.5 and 8.4 ± 2.5 (P < 0.01). Certain methods, particularly those based on ion-exchange HPLC, are reported to overestimate HbA_{1c} levels for patients with uremia (4,10). The mean difference in HbA_{1c} concentration determined between the Variant and Primus HPLC methods was 0.51 ± 0.42 for diabetic patients (n = 32) and 1.39 ± 0.89 for diabetic-uremic patients (n = 11), a significant difference (P = 0.005). Although the Primus HPLC has a very short 2-min turnaround time from specimen injection to display of the HbA_{1c} value, new assay sequences are initiated by two equilibration runs, therefore the first sample turnaround time is 6 min. Equilibration runs are necessary to effect a correct mobile phase balance between pH and salt concentration, and unless this occurs the first injection may yield erroneous results (R. Harrison [Primus Corp.], personal communication). As HbA_{1c} assays during AST predominantly involve single and small batch runs at irregular time intervals, we examined the relationship between HbA_{1c} value and time period from the last injection. The results established that without a prior equilibration run when the instrument had been idle for ~25–28 min, the measured HbA_{1c} value may be falsely elevated by ~30% (e.g., from 4.5 to 6.0%) because the glycated component does not achieve baseline resolution from the preceding large unglycated peak. Accordingly our AST protocol requires a blank run before specimen injection, whenever the HPLC idle time exceeds 20 min. No specimen showed a falsely elevated HbA_{1c} in the second sequential injection, therefore only one blank run is necessary. Performance within the actual outpatient setting was investigated during a 6-week period, with the Primus instrument relocated in close proximity to the blood room. In all cases patients' HbA_{1c} results were available before the actual clinic consult and before availability of blood glucose levels. The number of physicians in attendance at the clinic ranged from three to five. The DCA-2000 was evaluated in a similar manner but only for three clinic sessions, and two instruments were simultaneously used to maintain the assay throughput rate necessary. Limited comparison of the DCA-2000 (Y) and Primus (X) methods showed a bias of 0.7 (95% CI: 0.1 to 1.1) and a proportional error of −2.5% (95% CI: −6.3 to 4.8%) (Fig. 1); the corresponding mean HbA_{1c} levels for the sample set (n = 18) were 9.1 ± 2.5 and 8.6 ± 2.6 (P < 0.01).

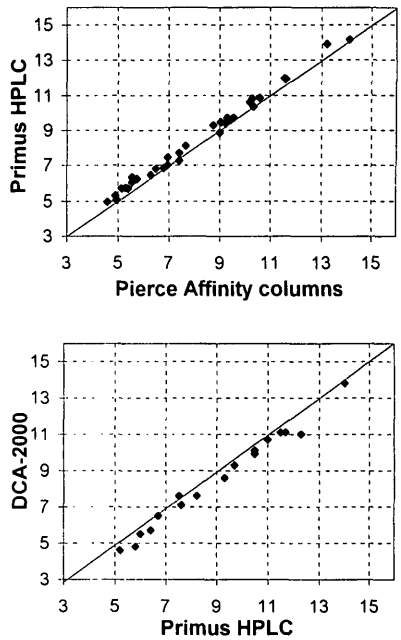

Figure 1—*Comparison of HbA_{1c} measurements: Pierce affinity mini-columns vs. Primus HPLC (upper) and Primus HPLC vs. DCA-2000 (lower). The line (y = x) represents equivalence between the methods.*

CONCLUSIONS — The Primus HPLC method for quantitation of HbA_{1c} concentration is a precise assay capable of exceptionally fast turnaround times and not subject to interference by carbamylated hemoglobin (4,10). The spurious elevation of HbA_{1c} results with the Variant HPLC for renal-diabetic patients, highlights the inadvisability of using ion-exchange HPLC procedures in a clinic which includes renal or uremic subjects. In Fig. 2 the turnaround assay times have been summarized for a single "stat" sample, on several different instruments. While the Variant HPLC method has a rapid 3-min analysis time, removal of the labile GHb (HbA_{1d}) requires at least 10 min. Although Bio-Rad has recently released an affinity column for the Variant HPLC, actual assay chromatographic time is considerably longer than that for the Primus, and results are only reported as GHb and not HbA_{1c}. The DCA-2000 is a portable, easily operated instrument, and not subject to the same

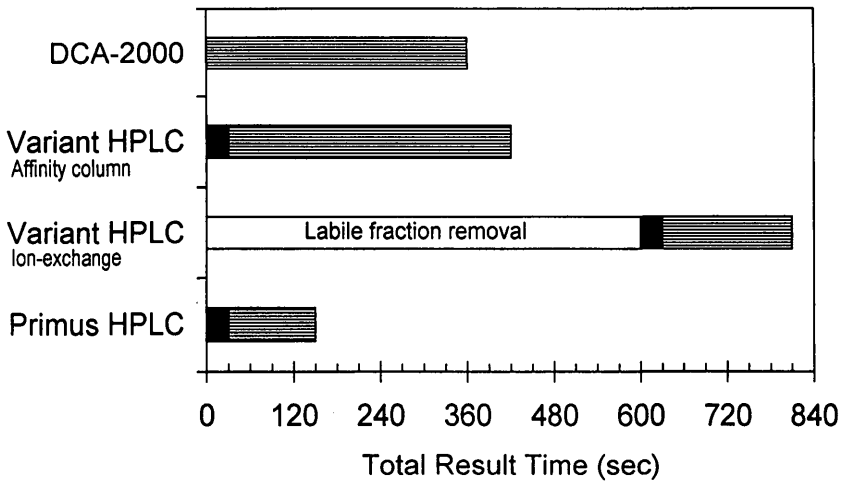

Figure 2—*Turnaround times for a single blood specimen on three different instruments assaying HbA_{1c} concentration. The blackened interval represents pre-injection processing time; the striped area is actual instrument turnaround time from injection until display of the HbA_{1c} level.*

interferences as the ion-exchange methods (4,10). To increase throughput of HbA_{1c} samples on the DCA- 2000, most AST sites use several instruments, since instrument cost is about 1/12 of that for an HPLC. In our limited study, AST during busy periods with two DCA-2000 instruments was more operator-demanding than that for the Primus HPLC. Reasons included the longer assay time (6 min), the care required in filling the cassette pipette, lack of a permanent record, and the potential for error through sample mix-up. Nevertheless the DCA-2000 has the advantage of minimum physical space requirements, portability, ease of use for nonlaboratory staff and the all-inclusive cartridge system. However, the DCA-2000 is unsuited to normal laboratory HbA_{1c} measurements, being labor-intensive and having negligible scope for connection to a laboratory information system. If different methods are used between AST and the main laboratory, then the reliability of serial HbA_{1c} changes is diminished. Notwithstanding such issues, the important question is whether there is an advantage to carrying out labor-intensive nonbatched HbA_{1c} assays in the clinic setting. A review of our outpatient booking records, over a 3-month period, established that 30% of physicians' time was related to viewing case notes. Subsequent discussions with the physicians revealed that a major part of this time related to following up HbA_{1c} results for patients seen during the last clinic. If major changes in glycemic control, not evident at the consult, were indicated by the HbA_{1c} value, the patients were either telephoned or scheduled for a revisit the following week. Consequently the availability of this same information at time of consult has the potential to save considerable human resources, not only clinical but also clerical, since the laboratory report no longer has to be entered into the patient case notes. The physicians considered the HbA_{1c} level the most important laboratory result required during the clinical assessment of their diabetic patients. In conclusion, the Primus CLC300 HPLC has the potential to deliver rapid HbA_{1c} results, with high precision and accuracy in an AST locality. Its actual sample turnaround time is considerably shorter than any other current method, and it also has the flexibility to automatically process very large numbers of HbA_{1c} specimens.

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