Liquid Chromatography of Glycated Hemobglobins with the Daiichi HA-8110 Automated Analyzer

To the Editor:

Measurement of glycated hemoglobins in blood hemolysates is considered useful for the assessment of long-term control of diabetes mellitus (1, 2). Recently, a fully automated instrument that can separate hemoglobin A (HbA) into its constituent fractions was introduced into Canada (Model HA-8110; Kyoto Daiichi Kagaku Co., Kyoto 601, Japan). In it the hemoglobin fractions are separated by liquid chromatography. The exact chemical nature of the column packing material is not disclosed by the manufacturer, but the separation principle is stated to be a combination of reversed-phase partition and cation-exchange chromatography on "Micropearl SF-W-A_{1c}" (Sekisui Kaguku Kogyo Co., Japan). Hemoglobin peaks are detected by bichromatic spectrophotometry and quantified by integration of peak areas. Hemoglobins A_{1a} and A_{1b} are not resolved by the instrument and consequently three peaks are obtained from most samples. Two correspond to the "fast" hemoglobin fractions (HbA_{1a+b} and HbA_{1c}) and the third to HbA_0. After each analysis, the chromatographic pattern is printed on a smaller printer that is built into the instrument, and a report is generated of the relative percentage composition of the hemoglobin fractions HbA_{1a+b}, HbA_{1c}, and HbA_0. The total HbA_1 percentage ($HbA_{1a+b} + HbA_{1c}$) is also printed.

During the evaluation, we used the instrument in accordance with the instructions of the manufacturer. Reagents were kindly supplied by Western Scientific Services, Rexdale, Ontario, the distributors of the instrument in Canada.

The procedure was as follows: Dilute 3 μL of whole blood containing EDTA as anticoagulant with 450 μL of hemolyzing reagent, using a diluter supplied with the instrument. Incubate the diluted samples for 5 h at 37 °C to destroy the labile fraction of glycated hemoglobins (3) and then load them on the instrument.

The instrument automatically samples the hemolysate and elutes the hemoglobins with two buffers, which differ in pH and salt concentrations. After each analysis is completed (13 min/sample), the results are printed.

We analyzed with this instrument samples from 194 patients who were attending a diabetic clinic. There was a good correlation between HbA_1 and HbA_{1c} [hemoglobin A_1 = (1.099 × hemoglobin A_{1c}) + 3.07; r = 0.955, n = 194]. This correlation is similar to that previously reported (4) and confirms that either HbA_1 or HbA_{1c} can be used to monitor diabetic patients. For 135 of the above samples, HbA_{1c} was determined manually by use of commercially available minicolumns (Bio-Rad Labs., Richmond, CA 94804). No incubation was required with this technique, because borate is used to destroy the labile glycated hemoglobins. Figure 1 depicts the correlation between results for HbA_{1c} obtained with the instrument and those with the Bio-Rad procedure; it was good, although the new method gave slightly higher results for HbA_{1c} than the manual procedure.
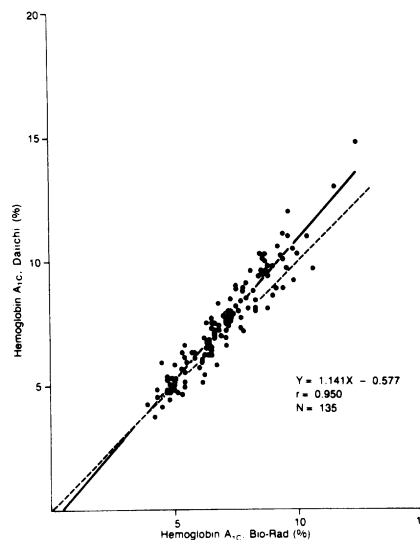

Fig. 1. Correlation of HbA_{1c} results obtained with the Daiichi HA-8110 Analyzer and the manual Bio-Rad minicolumn procedure

The *solid line* is the regression line, the *broken line* the line of equivalence

We assayed 46 samples for HbA_1 by use of a manual minicolumn procedure (Fast Hb Test System; Isolab Inc., Akron, OH 44821). With this method, labile glycated hemoglobins were destroyed by incubating the erythrocytes in isotonic saline for 48 h at 4 °C (5). Again, the correlation between methods was good, but the new method gave higher results for total A_1 than did the manual procedure [hemoglobin A_1 (Daiichi) = 0.992 × hemoglobin A_1 (Isolab) + 2.14; r = 0.949, n = 46].

Data on three patients, used to determine within-day precision of the instrument, are shown in the tabulation.

	HbA$_1$, %				HbA$_{1c}$, %		
n[a]	Mean	SD	CV, %	Mean	SD	CV, %
8	8.08	0.14	1.73	3.76	0.09	2.39
20	11.38	0.06	0.53	8.06	0.08	0.99
9	14.59	0.14	0.96	9.48	0.08	0.84

[a] No. of determinations.

Precision was excellent over a wide range of values. Because the instrument was available to use for only a short while, we were unable to assess between-day precision satisfactorily.

We also assayed with the instrument hemolysates containing abnormal hemoglobins. The abnormal hemoglobins were recognized by the microprocessor software as peaks that eluted earlier or later than the corresponding fractions of HbA; the chromatographic report was thus flagged as an "Abnormal Separation," and the unidentified peak areas were printed.

The automated HbA$_{1c}$ analyzer is simple to operate and maintain. Hemolysate preparation before analysis takes only a few minutes. The rate of analysis was only about four samples per hour, but this is not a major disadvantage because the instrument can be loaded with up to 50 samples at any one time and will operate unattended until the batch is completed. Our results support the findings of others (6), and suggest that the instrument should be considered as a potential alternative to more labor-intensive minicolumn procedures.

References

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