RAPID DESKTOP METHOD FOR THE MEASUREMENT OF GYLCATED HAEMOGLOBIN HBAlc

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ABSTRACT

A new desktop monoclonal antibody-based test system (Bayer Diagnostics DCA 2000™) for quantitation of glycated haemoglobin HbA1c is described and evaluated. This method involves a monoclonal antibody to HbA1c which defines its specificity. It requires 9 minutes to complete, and shows good intra- and inter-run precisions (1.3% – 3.7%), at 5 HbA1c levels tested – 4.1%, 5.7%, 6.1%, 9.2% and 12.4%. Results from 81 blood samples obtained from diabetic patients (3.9% – 13.2% HbA1c) showed excellent correlation with a laboratory-based ion-exchange HPLC technique (y = 1.03{Lab} + 0.103%; Pearson coefficient, r = 0.99). The test can be performed either with a capillary fingerprick or venous blood sample. Only 1µl of blood volume is required. Comparison of HbA1c levels of 43 paired capillary and venous samples showed excellent correlation (y = 1.00 {venous} + 0.042%; r = 0.99). The HbA1c values obtained from a cohort of 37 healthy adults, mean (± SD) age 33 ± 9.01 years, gave a value of 5.5 ± 0.42%. The calculated 95% confidence limits are 4.7% – 6.3%. This quick method provides 'stat' HbA1c results, which were hitherto not possible with the laboratory-based methods.

Keywords: *glycated haemoglobin A1c, DCA 2000, monoclonal antibody*

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INTRODUCTION

Regular measurement of haemoglobin A1 or A1c has assumed an important role in the treatment and control of diabetes[1,2]. It gives an accurate reflection of the mean blood glucose level over the preceding 2-3 months, and complements the practice of self-blood glucose monitoring. The current technologies for HbA1c measurements are laboratory-based, such as ion-exchange HPLC or affinity liquid chromatography. However, these are not "fast" enough for the clinician as an on-the-spot result which is available at the time of patient review, is desirable.

We report here an assessment of HbA1c quantitation using the DCA 2000™ analyser (Bayer Diagnostics, Germany). This analyser is part of the DCA 2000™ system which includes the reagent cartridges and reagent control (Fig 1). The test is based on latex immunoagglutination inhibition methodology. It has a mouse monocloncal anti-HbA1c antibody which is used in the latex agglutination inhibition step that ensured specificity of the test. An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c) causes agglutination of latex coated with HbA1c specific mouse monoclonal antibody. This agglutination reaction causes increased scattering of light which is measured as an increase in absorbance at 531 nm. HbA1c in whole blood specimens competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The HbA1c concentration is then quantified using a standard calibration curve of absorbance versus HbA1c concentration. All measurements and calculations are performed automatically by the DCA 2000™ Analyzer, and the screen displays percent HbA1c at the end of the assay.

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SUBJECTS AND METHODS

All tests were performed in accordance with the manufacturer's recommendations. Each lot of reagent kit contained reagent cartridges, capillary sample holders, calibration card and a temperature indicator card. The test procedure required that the calibration code for the particular batch be loaded into the analyser's memory, prior to use of reagent cartridges. Once the capillary sample holder is filled with blood sample, analysis must proceed within 5 minutes, by loading the cartridge into the analyser. Reaction buffer was released by removing (with a firm pull) the pull-tab. The machine gives a reading range of HbA1c concentration of 2.5% – 14.0 % in 9 minutes. It requires only 1µl of blood obtained either from a fingerprick capillary or venous blood sample. Patients were recruited from the outpatient Diabetic Clinic.

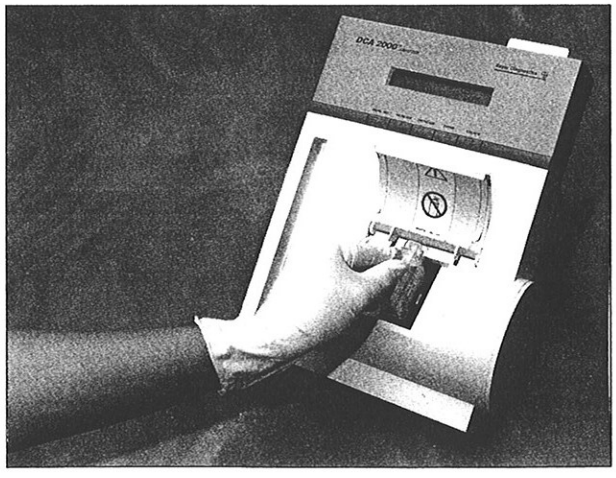
Assay Precision

Precision data were obtained from five venous EDTA-blood samples (range 4% – 12% HbA1c). Intra-CVs were calculated from test readings of 10 consecutive replicates. Inter-CVs were calculated from single readings obtained on 6-9 separate days.

Accuracy

Venous blood (n = 81) were collected in EDTA-tubes for immediate HbA1c quantitation with the DCA 2000™ analyser. A paired sample was despatched to the hospital's laboratory serv-

Fig 1 – DCA 2000™ analyser showing reagent cartridge being loaded.



ices for analysis with the ion-exchange HPLC technique (Diamat - Biorad Diagnostics Inc., USA)[3]. In addition, sample aliquots were also tested for total HbA1 with the boronate affinity chromatography technique (Glycotest II – Pierce Chemical Co., USA)[4].

Capillary and venous blood comparison

Comparison between capillary and venous blood samples for HbA1c were tested in 43 subjects.

Non-diabetic HbA1c levels

The normal reference range was derived from readings obtained from 37 healthy non-diabetic persons (16 male, 21 female). Venous blood samples were tested.

Statistical analyses

Results of continuous variables are given as mean (±SD) values. Appropriate statistics – linear regression, correlation and student's t tests, were applied where required.

RESULTS

Assay precision

The imprecision of the DCA 2000™ system for HbA1c determinations was <3% for intra-assay runs at 5 HbA1c levels ranging from 4.1% – 12.4%. Inter-assay CVs obtained over a 6-9 day period were equally good. Less than 5% were obtained at the 5 HbA1c levels (Table I). Similar imprecision variations were also obtained for the DCA 2000™ reagent controls of normal and abnormal levels (data not shown).

Table I – Precision data for HbA1c measurements with the DCA 2000 Analyser

	Mean HbA1c (%) ± SD	CV	
Intra-CV	4.1 ± 0.105	2.6%	
	5.7 ± 0.131	2.3%	
	6.1 ± 0.141	2.3%	
	9.2 ± 0.115	1.3%	
	12.4 ± 0.309	2.5%	n = 10
Inter-CV	4.3 ± 0.124	2.9%	
	5.5 ± 0.203	3.7%	
	5.8 ± 0.186	3.2%	
	9.4 ± 0.212	2.3%	
	12.3 ± 0.160	1.3%	n = 6 – 9

cv = coefficient of variation

Accuracy

The HbA1c percent concentrations determined by the DCA 2000™ analyser were accurate as compared with the laboratory-based ion-exchange HPLC technique (Diamat™). The Diamat™ instrument is an automated HPLC system – coefficients of variation (CV) for intra- and inter-assays were <3%[3]. With the DCA 2000™ system, HbA1c levels (n = 81) were very close to that determined by the laboratory technique (Fig 2, y = 1.03 {Diamat} + 0.103%, $p<10^{-6}$; Pearson coefficient, r = 0.99).

Fig 2 – Comparison of HbAlc results measured by DCA 2000™ with Diamat™

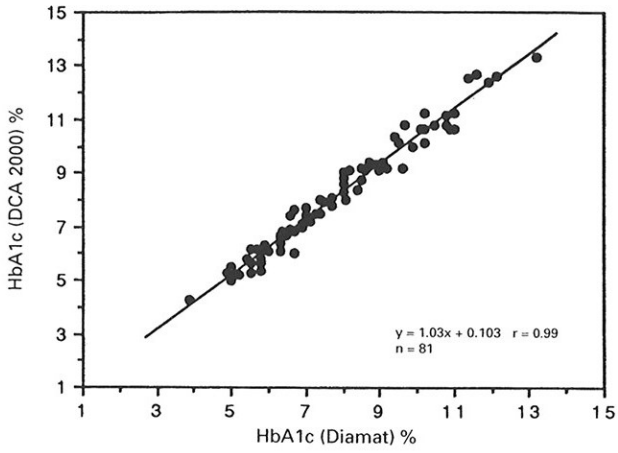


Fig 3 – Correlation of HbA1c (DCA 2000™) with total HbA1 (affinity chromatography) concentrations

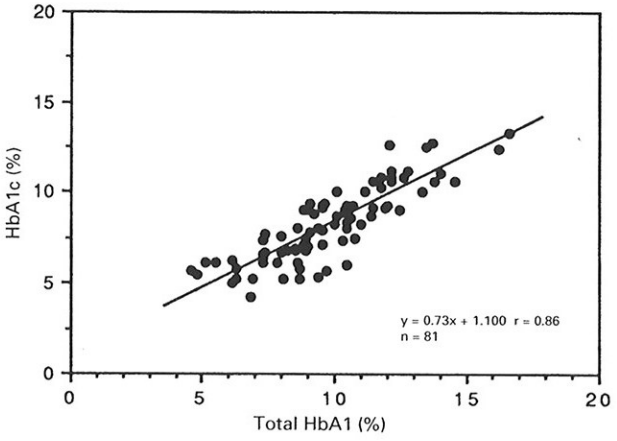


Fig 4 – Venous and capillary sample comparisons

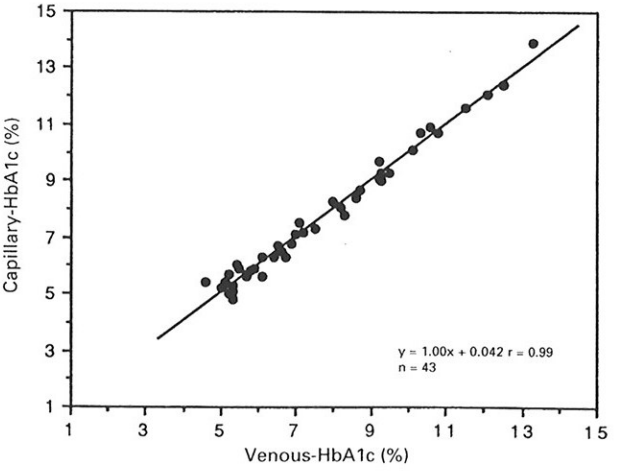


Table II – Accuracy of HbA1c measurements at different ranges

	Range	Mean HbA1c (%) ± SD		Linear Regression		Correlation	N
		Diamat	DCA 2000				
< 6.5%	3.9 – 6.4	5.65 ± 0.597	5.85 ± 0.626	DCA = 0.949 (Diamat)	+ 0.497	0.91	24
6.5 – 8.0%	6.5 – 8.0	7.26 ± 0.535	7.62 ± 0.733	1.197	– 1.076	0.88	22
8.1 – 9.0%	8.1 – 9.0	8.59 ± 0.287	8.96 ± 0.429	1.030	+ 0.113	0.79	12
> 9.0%	9.1 – 13.2	10.49 ± 1.060	10.84 ± 1.173	1.016	+ 0.171	0.92	23
entire range	3.9 – 13.2	7.89 ± 2.034	8.21 ± 2.119	1.026	+ 0.103	0.99	81

Further analysis of the values determined by DCA 2000™, showed that linear regression statistics were also extended to different levels within the entire measured range 4 – 14% (Table II). However, a lower correlation with total HbA1 determined by boronate affinity method, was obtained (Fig 3, r = 0.86).

Capillary and venous blood comparison

Capillary and venous blood HbA1c from 43 subjects were compared. The results indicated that HbA1c levels for both capillary and venous blood were identical (Fig 4, y = 1.00 {venous} + 0.042%, $p<10^{-6}$; r = 0.99). No statistical differences were indicated for the mean HbA1c levels of capillary (7.7±2.33%) and venous blood (7.6±2.31%).

Non-diabetic HbA1c levels

A normal HbA1c reference range was obtained from 37 healthy persons (16 male, 21 female). Their overall mean age was 33±9.0 years (range 22 – 50 years). Haemoglobin A1c levels obtained from them, ranged from 4.6 – 6.5%, giving a mean HbA1c value of 5.5 ± 0.42%. The calculated 95% confidence limits would be 4.7% – 6.3%.

DISCUSSION

The desktop DCA 2000™ analyser for HbA1c measurements is accurate and precise as shown in this study. Favourable inter-CVs were obtained for up to 9 days. The non-diabetic reference range of HbA1c falls within a 95% confidence interval of 4.7% and 6.3%. Taking only 9 minutes to complete, the test is easy to perform and requires no pre-treatment of sample for analysis.

Additionally, there is little dependence on operator competence though it is important to ensure that the capillary sample holder is completely filled. Abnormal haemoglobin concentrations from anaemia or incomplete filling of sample holder, would automatically result in an aborted test run. The test is specific for the beta-chain of HbA and hence is not affected by haemoglobin variants such as HbS and HbC. However, samples containing high amounts of HbF (>10%) may yield lower than expected haemoglobin A1c results with this test. The labile fraction (Schiff base or aldimine) also does not affect the assay since the antibody is specific for the stable ketoamine. This method therefore has none of the usual limitations of the current commonly used methods for HbA1 analysis, eg mini-column chromatography and electrophoresis[5-8]. The DCA 2000 HbA1c assay gives accurate and precise results over a range of total haemoglobin of 7 to 24 g/dL. With severe anaemia and polycythemia, patients should be assayed by a test employing a different assay principle if their haemoglobin concentrations are outside of the acceptable range.

The National Institute of Health Diabetes Data Group has set guidelines for the measurement of HbA1c[9]. They recommended that, without an agreement on either a reference method or HbA1c standard, the inter- and intra-assay imprecision should be less than 5%. The normal reference range should be narrow (≤ 2%) and the method should be for HbA1c fraction as it appears to be the only component of consequence with respect to diabetes mellitus. The DCA 2000™ system for HbA1c measurement therefore would meet these recommended guidelines.

The DCA 2000™ system's analytical characteristics for HbA1c quantitation also appear to be much better than those for another monoclonal antibody-based test system, an enzymeimmunoassay (EIA) by Novo Biolabs[10,11]. The EIA technique has a wider variance for test results when related against those obtained by HPLC (Pearson coefficient r = 0.95) compared to the DCA 2000™ (r = 0.99) This is the only other method employing specific immunochemical reactions for the measurement of HbA1c. However, it is noted that the EIA test is a batch-type assay and would be difficult to accommodate 'stat' measurements. Indeed, the DCA 2000™ system is the 'fastest' test currently available, for the quantitation of HbA1c. The use of capillary blood samples obviates the need for venepuncture, since a single drop of blood from a fingerprick will suffice. Its fast assay time allows for quick reporting of HbA1c results to the clinician. This is an advantage as the immediate information regarding glycaemic control allows the clinician to make objective therapeutic decisions at the same patient visit. Another useful feature is that it is a simple technical procedure using a compact desktop machine which can be operated by non-laboratory personnel. This new method would thus be suitable for small to medium sized outpatient clinics, either hospital-based or in the general physician practice.

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