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ORIGINAL ARTICLE

Performance evaluation of the Arkray Adams HA-8160 HbA_{1C} analyser

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Abstract

Background: HbA_{1c} measurement is currently routinely used to predict long term outcome of diabetes, thus playing a fundamental role in the management of diabetes. The relationship between HbA_{1c} value and long term diabetic complications has been established by a randomised control Diabetes Control and Complications Trial (DCCT) which used high performance liquid chromatography (HPLC) as a reference method for HbA_{1c} assay. To ensure that HbA_{1c} results from a variety HbA_{1c} assay methods are similar to the DCCT values, the American Diabetes Association (ADA) recommended that all laboratories should use methods certified by the National Glycohemoglobin Standardization Programme (NGSP) with interassay coefficient variation (CV) of < 5% (ideally < 3%). The International Federation of Clinical Chemistry (IFCC) working group on HbA_{1c} standardisation has set a CV < 2.5% as a criteria for its reference laboratories. *Objectives:* To evaluate the performance of Arkray Adams HA-8160 HbA_{1c} analyser which uses a cation exchange HPLC method and its correlation to HbA_{1c} assay on Cobas Integra 800 which is an immunoturbidimetric method. *Methods:* For the imprecision study, patient samples and control material of two levels were analysed on HA-8160 analyser 20 times in a single run (within-run imprecision) and twice a day on five consecutive days (between-run imprecision). For the recovery study, two samples each with high and low values were selected and mixed in ratios of 1:3, 1:1 and 3:1, and were analysed by HA-8160. Sixty samples were analysed by both Cobas Integra 800 and HA-8160 for method comparison study. Ten uraemic samples and ten thalassaemic samples were assayed on Cobas Integra 800 and HA 8160 for interference study. *Results:* Within-run CVs were 0.6% and 0.7% for medium and high value samples respectively, 0.6% and 0.7% for low and high level controls respectively. Between-run CVs were 0.5% and 0.4% for medium and high value samples respectively, 0.5% and 0.6% for low and high level controls respectively. The mean recovery was 100.1%. A good correlation between the 2 methods (Adams = 1.00 Cobas - 0.11, r = 0.98) was observed. *Conclusions:* The Akray Adams HA-8160 HbA_{1c} analyser performed within the target CV of < 2.5% and showed a good correlation with the Cobas Integra 800.

Keywords: diabetes, HbA1c, HPLC

INTRODUCTION

Estimation of glycated haemoglobin (GHb), particularly HbA_{1c}, as an index of glycaemic control is fundamental to the management of diabetes. Prospective randomised clinical trials such as the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have determined the relationship between specific HbA1c values and long term outcome risks in diabetic. The results of DCCT have been used as a platform for establishing specific treatment goals using glycated haemoglobin as an index of mean glycaemia. The American Diabetes Association (ADA) recommends that GHb should be maintained at < 7% to decrease the risk of development of long term complications of diabetes mellitus. Re-evaluation of the treatment regime should be considered with repeated GHb > 8%.[1,2,3]

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HbA_{1c}

The International Federation of Clinical Chemistry (IFCC) defines HbA_{1c} as haemoglobin that is irreversibly glycated at one or both N terminal valines of the β-chains.[4] HbA1 is a fast migrating component of haemoglobin detected on gel electophoresis. The N-terminal valine of the β-chain provides the most common site of glycation within the haemoglobin tetramer accounting for 80% of HbA1.[5] The glycation process involves a two-step reaction of glucose with adult haemoglobin (HbA). The first step is a reversible enzymatic reaction between glucose and haemoglobin forming a labile Schiff base while the second step is an irreversible non-enzymatic Amadori rearrangement that produces GHb.[5] The labile schiff base interferes with some HbA_{1c} assay methods.

HbA_{1c} Assay standardisation

There are many different HbA_{1c} assay methods in current use. Assay principles are based on charge differences between glycated and non-glycated components (High Performance Liquid Chromatography (HPLC), electrophoresis) or on structural differences (immunoassays, affinity chromatography).[5,6] For the optimum use of GHb tests, many programs were initiated and authorised bodies were formed to achieve standardisation of these methods. The National Glycohaemoglobin Standardization Program (NGSP) was initiated in 1996 to standardise HbA_{1c} results among laboratories to be comparable to DCCT values. The DCCT method is an HPLC cation–exchange method and is a National Committee for Clinical Laboratory Standards (NCCLS) designated comparison method.[7] Currently, ADA recommends that all laboratories should use only methods that are certified by the NGSP with interassay coefficient of variation (CV) of < 5% (ideally < 3%).[1,7,8] For global standardisation, the IFCC has established a working group to develop new reference methods based on mass spectrometry or capillary electrophoresis and highly purified calibrator with reference CV < 2.5%.[9,10] It has been agreed that all HbA_{1c} analysers will be calibrated against the IFCC reference method.

Interference of HbA_{1C} assay

Any condition that shortens erythrocyte survival (haemolytic anemia, haemoglobinopathies) or decreases mean erythrocyte age (recovery from blood loss) can falsely lower HbA_{1c} results and conditions that prolong erythrocyte survival (e.g. iron deficiency anemia and splenectomy) may falsely increase HbA_{1c} results.[1,6,7] Haemoglobin variants (HbS, C, D), foetal haemoglobin (HbF) and carbamylated haemoglobin (in uraemia) interfere with some assay methods.[11,12] ADA recommends that the laboratories should be aware of potential interferences that may affect HbA_{1c} test results and consider the potential for interferences in their particular patient population when selecting assay methods.[1]

We evaluated the analytical performance of a new Arkray Adam HA-8160 HbA_{1c} analyser which uses an HPLC cation exchange method and correlated it to the current HbA_{1c} analyser used in our laboratory (Cobas Integra 800) which uses an immunoturbidimetric method.

MATERIALS AND METHODS

Tests were run on the Adam's HA-8160 HbA_{1c} analyser using Diabetic Monitoring mode. The analyser currently used in our laboratory (Cobas Integra 800) was used as a comparison method.

Sample storage

All samples except for samples for between-run study were stored at room temperature (25º C) and run within 24 hours from time of sample collection. Samples for between-run study were stored at 8º C for a maximum of 5 days.

Imprecision

For within-run study, two levels of commercial controls, i.e. Bio-Rad Lypocheck Diabetes Level 1(low control) and Level 2 (high control), and two patient samples at two levels (high and low) were used. The patient samples were assayed 20 times on the HA-8160 analyser in a single run concurrently with the control materials which were run in duplicates. All samples were run as whole blood. For between-run study, the control material and two patient samples at two levels were run in duplicate every morning and afternoon for five consecutive days. All samples were run as haemolysate.

Recovery

Two patient samples with high and low values were run five times to obtain neat high and neat low values. These samples were then mixed in ratios of 1:3, 1:1 and 3:1. The three mixtures were run in duplicate. Expected HbA_{1c} values were calculated for each mixture using neat high and neat low value. Percentage of recovery was calculated using HbA_{1c} values for each mixture. All samples were run as haemolysate.

TABLE 1: Within-run imprecision of Adam's HA-8160 HbA$_{1c}$ analyser

Patient samples		Low level (n=20)	High level (n=20)
	Mean HbA$_{1c}$ (%)	6.61	12.9
	Within-run CV (%)	0.60	0.7
Controls		Level 1 (n=20)	Level 2 (n=20)
	Mean HbA$_{1c}$ (%)	5.39	9.13
	Within-run CV (%)	0.6	0.7

Method comparison
Sixty patient samples of various levels were analysed on both Cobas Integra 800 and Adam's HA-8160 HbA$_{1c}$ analyser. All samples were analysed as whole blood.

Interference study
Ten samples from uraemic patients and ten samples from thalassaemic patients were assayed on both Cobas Integra 800 and HA-8160. Out of the 10 uraemic patients, 6 had undergone haemodialysis and 1 had received blood transfusion. Only 1 out of 10 thalassaemic patient had received blood transfusion. All samples were run as whole blood.

HbA$_{1c}$ assay methods
The Cobas Integra 800 (Roche Diagnostics, Penzberg, Germany) is a fully automated HbA1c analyser using an immunoturbidimetric method with a colorimetric assessment of total haemoglobin. HbA$_{1c}$ is measured using monoclonal antibodies bound to latex particles that react with β-N terminal fragments of HbA$_{1c}$. The remaining free antibodies are agglutinated with a synthetic polymer carrying copies of the β-N terminal structure of HbA1c. The change in turbidity is inversely proportional to the HbA$_{1c}$ concentration. The final result is expressed as percentage of total haemoglobin.

The Adams HA-8160 (Arkray KDK, Kyoto, Japan) HbA$_{1c}$ analyser is a fully automated HbA$_{1c}$ analyser using reversed-phase cation exchange chromatography with colorimetric method of detection (measured at wavelength of 415 nm and blanking wavelength of 500 nm). The sample volume used was 4μl of EDTA whole blood which was automatically haemolysed by the analyser. Results are presented as % HbA$_{1c}$, % HbA1 and %HbF which are calculated from the peak areas of different hemoglobin fractions as a percentage of total hemoglobin.

Data analysis
All statistical analyses were performed using Microsoft excel. Coefficients of variation were calculated for the imprecision study. Deming linear regression was used to analyse method comparison. Bland-Altman plot was generated from method comparison data using MedCalc statistical software.

RESULTS

Data from the imprecision study is summarised in Tables 1 and 2. HA-8160 showed good precision with CV < 2.5% for both controls and samples. Good recovery with mean of 100% was obtained

TABLE 2: Between-run imprecision of Adam's HA-8160 HbA$_{1c}$ analyser

Patient samples		Low level (n=20)	High level (n=20)
	Mean HbA$_{1c}$ (%)	6.49	10.69
	Between-run CV (%)	0.5	0.4
Controls		Level 1 (n=20)	Level 2 (n=20)
	Mean HbA$_{1c}$ (%)	5.41	9.26
	Between-run CV (%)	0.6	0.6

TABLE 3: Deming linear regression parameters for y = mx + c, mean bias and Pearson correlation.

x	y	Slope (m)	Intercept (c)	Mean bias (y-x)	R
Cobas Integra 800	Adam's HA-8160	1.05	-0.11	-0.07	0.98

by using the analyser. Results for analysis of method comparison study are summarised in Table 3 and Bland –Altman plot is shown in Figure 1. HA-8160 showed good correlation with Cobas Integra 800 with HA8160 = 1.00Cobas 800 – 0.11 (r =0.98). Bland-Altman plot of HA-8160 against Cobas Integra 800 produced a mean bias of -0.07 (-0.57- 0.43 lower and upper 95% confidence interval). Results from the interference study are shown in Table 1V (uraemic effect) and Table 5 (thalassaemic effect). Both Cobas Integra 800 and HA-8160 obtained low HbA_{1c} values with mean of 6.37% and 6.4% respectively for uraemic samples and mean of 5.1% and 5.2% respectively for thalassaemic samples.

DISCUSSION

In keeping with increasing demand for HbA_{1c} measurement in the management of diabetes, the need for efficient and reliable GHb assay methods has become essential. We evaluated the analytical performance of Arkray Adam HA-8160 HbA_{1c} analyser which is based on HPLC aiming for a CV of < 2.5% as specified by the IFCC working group for HbA_{1c} standardisation. We have also compared this method with the immunoturbidimetric method adopted by Cobas Integra 800 analyser currently used in our laboratory.

The Adam's HA-8160 HbA_{1c} analyser showed good within and between-run imprecision which fulfils with our target CV of < 2.5%. However, it should be noted that the between-run imprecision results probably underestimate the true imprecision of the system because it was assessed for only five days rather that twenty days as recommended by the NCCLS.[13] This may explain the lower value of between-run CVs compared to that of within run CVs obtained from this study. The imprecision of the Cobas Integra 800 was not assessed during the evaluation. A CV of 1.7 was documented for Cobas Integra

TABLE 4: Effect of urea on HbA_{1c} using Cobas Integra 800 and Adams HA-8160 HbA1c analyser

No	Hb (g/L)	Urea (mmol/L)	HbA_{1c} (%)	
			Cobas Integra 800 (n=10)	Adams HA-8160 (n=10)
1	88	19.0	7.36	7.6
2	101	20.6	5.76	5.8
3	115	32.0	9.18	9.3
4	111	33.5	5.29	5.3
5	92	26.0	6.72	6.7
6	83	13.5	5.35	5.5
7	59	45.0	6.37	6.3
8	93	40.0	5.86	6.0
9	96	36.0	5.56	5.7
10	99	41.1	6.23	6.1
MEAN		30.7	6.37	6.4

TABLE 5: Effect of HbF and HbA2 on HbA$_{1c}$ using Cobas Integra 800 and Adams HA-8160 HbA$_{1c}$ analyser

No	(g/L)	Hb (%)	Hbf (%)	HbA$_{1c}$ (%)	
				Cobas Integra 800 (n=10)	Adam's HA-8160 (n=10)
1	78	4.9	0.2	5.62	5.7
2	113	30.2	0.5	6.11	5.4
3	124	6.0	2.3	5.27	5.6
4	87	5.8	3.1	4.96	5.0
5	105	8.3	5.7	5.14	5.3
6	90	5.7	1.5	6.00	5.9
7	124	6.1	2.8	4.89	5.2
8	104	6.3	1.1	4.55	4.7
9	118	6.4	5.2	4.47	4.9
10	107	29.5	0.5	4.07	4.7
MEAN				5.1	5.2

800 from external quality assessment done at the closest time to the evaluation which suggests that HA-8160 is more precise. However, this may be misleading because the CV for Cobas Integra 800 was obtained using different samples and different protocol of method evaluation. The accuracy of the analyser is excellent with a mean recovery of 100%. Good agreement (r = 0.98) between HA-8160 and the Cobas Integra 800 was observed.

It has been established that haemoglobinopathies and chemically modified derivatives of haemoglobin, i.e. carbamyl Hb in uraemic samples, interfered with some GHb assay methods either positively or negatively.[12] Bry *et. al*., in his study comparing three methods of HbA$_{1c}$ assays (immunoassay, cation exchange chromatography and boronate affinity) concluded that HbF concentration of < 5% of total haemoglobin has no significant effect on the

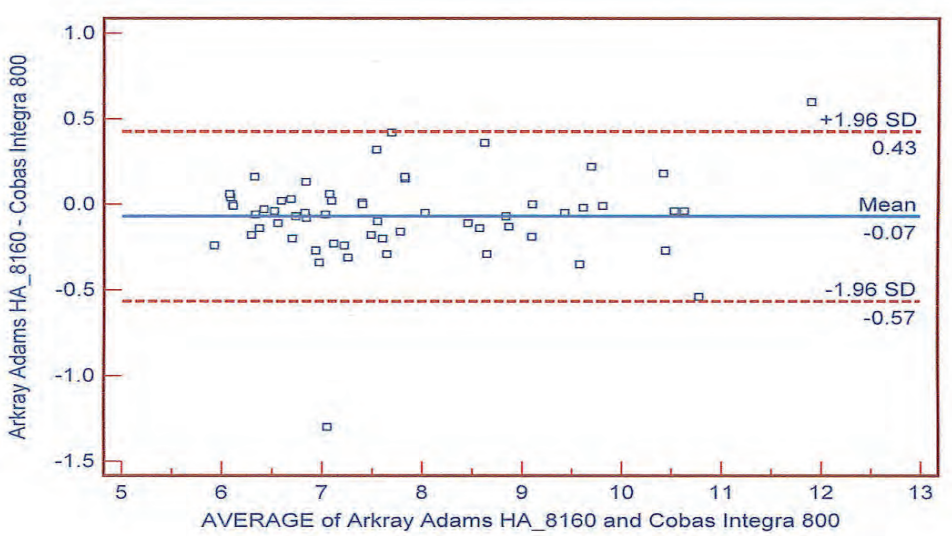

FIG. 1: Bland –Altman plot of Adams HA-8160 method against Cobas Integra 800 method

majority of GHb assay methods.[8,14] Studies have also shown that most cation exchange chromatographic methods separate HbF and glycated HbF from HbA and HbA_{1c} allowing accurate determination of HbA_{1c}.[11,14,15,16] The manufacturer of the Cobas Integra 800 claims that HbA_{1c} values may be underestimated if the sample contains HbF > 10% and carbamylated Hb does not interfere with the test. Therefore, one can assume that the results obtained by the Cobas Integra 800 in the interference study are free from interference. The results obtained by HA-8160 are comparable to those obtained by the Cobas Integra 800 which may suggest that HbF and carbamylated Hb do not interfere with HA-8160 HbA_{1c} assay. Increased in HbF peak can be seen well in advance of the HbA_{1c} from the chromatogram produced by HA-8160 analyzer.

No conclusion regarding the interference of haemoglobin variant (HbF and carbamylated Hb) can be drawn from this evaluation due to the small number of samples evaluated (n =10 for each condition). However, both analysers in both conditions obtained low HbA_{1c} values. It should be noted that most of the uraemic samples were also anaemic and some patients might be on haemodialysis which shortens the red cell life span and may contribute to HbA_{1c} interference. The ability of HA-8160 to report HbF percentage and availability of chromatogram in the report may alert the laboratory staff to the presence of a haemoglobin variant or possible interference.

Overall, HA-8160 analyser is very simple, easy to operate and efficient. The time for first result was 4.6 minutes and 2.9 minutes for subsequent results that gives a throughput of 20 tests per hour. It can hold up to 100 samples at one time and allows continuous loading while running. On the spot STAT measurement is available and only a small volume of sample is required (dead volume of 1 ml). The analyser software allows searching or tracing of previous data. It has sensor levels to prevent reagent shortages during measurement and no calibration is required when the reagent is changed. For maintenance, users are required to change prefilter every 500 samples to increase the life span of the column. However, the manufacturer recommends column change for every 2500 to 3000 samples. No major down time problem that required backup from the manufacturer occurred during the short evaluation period.

In conclusion, the Arkray Adam's HA-8160 analyser is a reliable HPLC-based HbA_{1c} analyser with acceptable imprecision, good accuracy and efficiency. It is comparable to our current immunoturbidimetry-based analyser (Cobas Integra 800) and is, thus, an excellent alternative to the Cobas Integra 800.

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