Performance evaluation of ARKRAY HA-8190V system for measuring glycated hemoglobin

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ABSTRACT

Introduction: the new fully automated HPLC ion-exchange system ADAMS A1c HA-8190V analyzer, developed by ARKRAY Inc., running in two different modes (Variant and Fast) has been evaluated.
Methods: reproducibility was evaluated according to the EP-15A3 standard. Method comparison was performed on 122 fresh blood samples, according to the EP-9 standard. The system was compared to 3 other HPLC analyzers, based on ion-exchange (Tosoh G11 and Bio-Rad D-100) and boronate affinity chromatography (Trinity Biotech Premier Hb9210). Usability was evaluated by using a score evaluation system.
Results: reproducibility proved to be very good at normal and high HbA_{1c} concentration, with total CVs always <0.7 %, when HbA_{1c} was expressed in mmol/mol as well as in % units. The HA-8190V system was well correlated to the other HPLC analyzers, with a mean bias not clinically relevant. Finally, the usability of the system was evaluated and proved to be well acceptable.
Conclusions: the ARKRAY HA-8190 V system was found to be a reliable and suitable method for routine HbA_{1c} measurement in clinical chemistry laboratories.

Keywords: *HbA1c, diabetes, boronate affinity chromatography*

INTRODUCTION

Glycated hemoglobin (HbA_{1c}) testing has a central role in the evaluation and management of diabetes mellitus. Traditionally, it is used to monitor the retrospective glycemic control of patients, to evaluate the risk of developing long-term complications and to make decisions concerning therapy. More recently, it is also used for the diagnosis and screening (1,2), as well as an important step in screening for gestational diabetes (3).

The importance of HbA_{1c} in diabetes management is well recognized by the diagnostic industry and many commercial methods are available to determine the HbA_{1c} concentration. They are essentially based on two principles, one consisting in electric charge differences (ion-exchange HPLC, capillary electrophoresis) and the other in structural differences (boronate affinity chromatography, immunochemical and enzymatic assays) between HbA_{1c} and other non-glycated forms of hemoglobin (4).

Whatever the assay method used, it is required to be standardized and to achieve a high degree of analytical performance, possibly near to the recommended goals (5), due to the important clinical decisions based on HbA_{1c} results. Moreover, it is known from epidemiological data that the diabetes prevalence has been rising in recent decades worldwide (6), so the usability characteristics of the analytical systems are important issues to take into account because of the increasing number of tests daily requested to the laboratories.

ARKRAY HA-8190V is a recently developed HPLC

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analyzer for the measurement of HbA_{1c}. We have performed an evaluation of the main analytical performances of this new instrument and report here the results of the reproducibility and method comparison studies as well as some usability aspects.

METHODS

ADAMS A1c HA-8190V

HA-8190V is a fully automated bench-top HbA_{1c} analyzer using the principle of cation-exchange HPLC for HbA_{1c} separation and quantification. The instrument has two modes of operation for the measurement of HbA_{1c}, the Fast mode (runtime 24 s; variant hemoglobins are not separated) and the Variant mode (runtime 58 s; variant hemoglobins are separated). Switching between the two modes does not need the change of any reagents or column. A two-points calibration is used, and results are reported in both mmol/mol and % units. A typical separation pattern is shown in Figure 1.

Samples

Patient samples consisted of EDTA anticoagulated blood samples, freshly collected from left-over material of the laboratory HbA_{1c} routine assay and anonymized before their use. They were tested using Bio-Rad D-100 and Hb variant blood samples were excluded from this study. Quality control and calibration materials were provided by the manufacturers of the different tested analyzers and used according to their instructions.

Reproducibility study

The reproducibility study was performed according to CLSI EP-15A3 standard. Over five consecutive days, two control materials (level 1 and level 2 supplied by ARKRAY), together with three whole blood pools having increasing concentrations of HbA1c (pool 1: physiological level; pool 2: moderately high; pool 3: high) were analyzed. The three blood pools were prepared by mixing fresh blood samples. Once prepared, they were divided in aliquots and stored at -80 °C until the analysis.

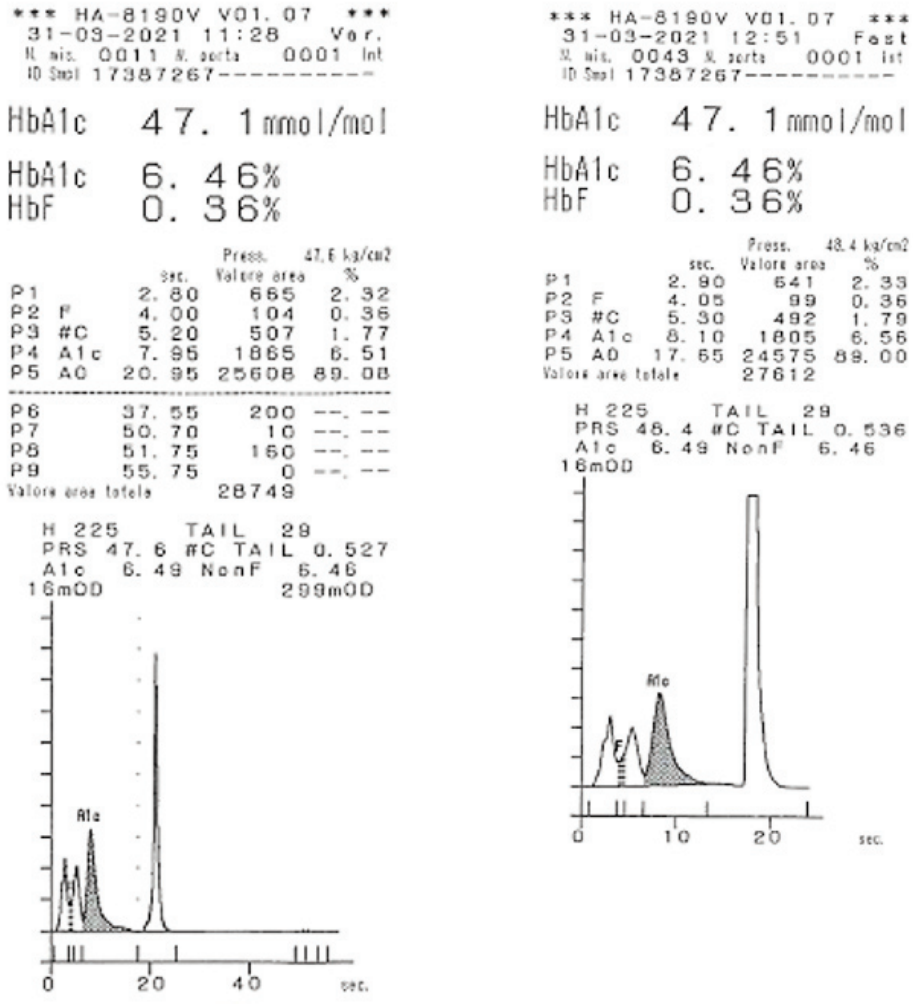

Figure 1
Typical chromatograms obtained, for the same blood sample, using HA-8190V operating in Variant (left) and Fast (right) mode.

The tests were carried out in both Variant and Fast mode.

Method comparison study

HbA_{1c} results obtained with HA-8190V using both Variant and Fast mode, were compared to the results obtained with three different HPLC systems, i.e. Bio-Rad D-100 and Tosoh G11 both based on ion-exchange chromatography and Trinity Premier Hb-9210 based on boronate affinity chromatography.

A total of 122 blood samples, selected to be distributed across the entire physio-pathologic HbA_{1c} range, were used for the comparison study. Blood samples were stored at 4 °C until the analysis was performed within 24-48 hours from the collection. The comparisons were carried out in several analytical sessions by analyzing a series of 30 blood samples over a period of about one month. At the beginning and at the end of each analytical session, two control materials (at low and high HbA_{1c} levels) supplied by the manufacturers of all the tested HPLCs were analysed. All the methods were run and calibrated according to the manufacturer's instructions. In particular, for ARKRAY HA-8190V, Bio-Rad D-100 and Tosoh G11 it was carried out only once at the beginning of the comparison study, while for Trinity Premier Hb9210 at the beginning of each analytical session.

Usability

Usability has been evaluated by two independent users, taking into account automation and user interface, throughput, calibration requirements, sample and reagents management, maintenance needs and the overall user-friendliness of the system. A score was given to the various aspects of the evaluation, by making a comparison with the other systems used in this study.

Statistical analysis

The results of the reproducibility study were elaborated by the ANOVA analysis. The results of the method comparison study were evaluated by using the non-parametric regression model according to Passing-Bablok and the Bland-Altman analysis. The statistical package MetComp ver. 1.0 (SIBioC, Italy) was used for all statistical tests.

RESULTS

Reproducibility

The reproducibility parameters as investigated by CLSI EP-15A3 protocol are listed in Table 1. For results expressed in mmol/mol (Table 1A) and operating in Variant mode, an excellent reproducibility was found with within-run CVs of 0.5 - 0.7 %, between-run CVs of 0.2 - 0.4% and

Table 1
Reproducibility of HbA_{1c} assay by ADAMS A1c HA-8190V operating in Variant and Fast mode.
CV values were calculated from HbA_{1c} results expressed in SI (A) and in % (B) units.

A	Control L	Control H	Pool 1	Pool 2	Pool 3
Variant mode					
Mean (mmol/mol)	33.76	102.92	36.21	55.12	76.15
CV-within run (%)	0.59	0.56	0.67	0.45	0.51
CV-between run (%)	0.35	0.31	0.28	0.23	0.37
CV total (%)	0.63	0.58	0.66	0.47	0.58
Fast mode					
Mean (mmol/mol)	31.61	98.11	35.80	55.05	76.05
CV-within run (%)	0.50	0.28	0.30	0.30	0.21
CV-between run (%)	0.45	0.32	0.41	0.21	0.17
CV total (%)	0.64	0.41	0.49	0.34	0.26
B	Control L	Control H	Pool 1	Pool 2	Pool 3
Variant mode					
Mean (%)	5.24	11.57	5.46	7.19	9.12
CV-within run (%)	0.31	0.45	0.40	0.31	0.38
CV-between run (%)	0.17	0.23	0.18	0.17	0.30
CV total (%)	0.33	0.47	0.40	0.33	0.45
Fast mode					
Mean (%)	5.04	11.13	5.43	7.19	9.11
CV-within run (%)	0.32	0.22	0.17	0.22	0.16
CV-between run (%)	0.28	0.25	0.20	0.15	0.12
CV total (%)	0.40	0.32	0.26	0.25	0.19

a total imprecision between 0.5 - 0.7%. Using the Fast operation mode, the reproducibility was even better, with total CVs between 0.3 and 0.6%. The CV values obtained for results expressed in % units are detailed in Table 1B.

Method comparison

The linear regression parameters from Passing & Bablok analysis together with the mean bias obtained in the comparison studies are shown in Table 2.

Bland-Altman plots of differences between the HA-8190V and the other tested HPLC methods are shown in Figure 2.

Table 2

Passing-Bablok regression parameters and mean bias (method y – method x) calculated from the method comparison study for HbA1c assay expressed in SI (A) and in % (B) units.

A	Method x	Method y	n	Intercept (95% CI)	Slope (95% CI)	Mean bias (95% CI)
	Bio-Rad	ARKRAY V	122	0.527 (-0.257÷1.290)	0.998 (0.980÷1.014)	0.50 (0.25÷0.74)
	Tosoh	ARKRAY V	122	-0.292 (-0.875÷0.265)	1.004 (0.992÷1.018)	0.00 (-0.20÷0.20)
	Trinity	ARKRAY V	121	-1.457 (-2.335÷-0.567)	0.987(0.967÷1.006)	-2.05 (-2.37÷-1.74)
	ARKRAY F	ARKRAY V	122	-0.349 (-0.617÷-0.050)	1.007 (1.000÷1.012)	-0.05 (-0.13÷0.03)
B	Method x	Method y	n	Intercept (95% CI)	Slope (95% CI)	Mean bias (95% CI)
	Bio-Rad	ARKRAY V	122	0.054 (-0.054÷0.161)	0.998 (0.980÷1.014)	0.05 (0.02÷0.07)
	Tosoh	ARKRAY V	122	-0.035 (-0.118÷0.042)	1.004 (0.992÷1.018)	0.00 (-0.02÷0.02)
	Trinity	ARKRAY V	121	-0.106 (-0.226÷0.020)	0.987(0.967÷1.006)	-0.19 (-0.22÷-0.16)
	ARKRAY F	ARKRAY V	122	-0.046 (-0.082÷-0.005)	1.007 (1.000÷1.012)	-0.01 (-0.01÷0.00)

CI, Confidence interval; F, Fast mode; V, Variant mode.

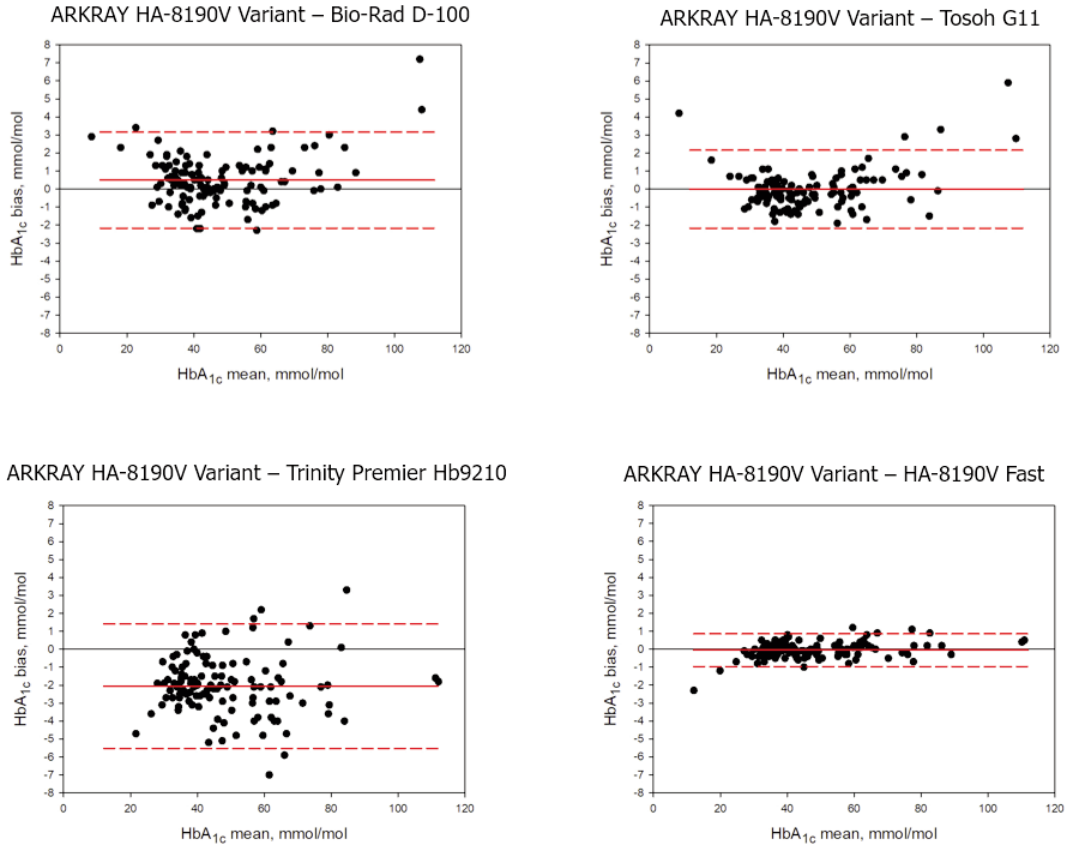

Figure 2

Bland-Altman difference plots showing the absolute bias between HbA_{1c} results obtained with HA-8190V Variant mode and the other tested methods. On the x-axis, the average of HbA_{1c} values obtained with the couples of methods is reported. Mean bias (red solid line) and 95% range of differences (dashed lines) are indicated.

The results obtained with the HA-8190V operating in Variant mode were almost equivalent to those obtained in Fast mode, also considering that no hemoglobin variants were present in the blood samples. For this reason, only the results obtained with the Variant mode were used for the comparisons.

The HA-8190V was perfectly aligned to both Bio-Rad D-100 and Tosoh G11 systems, as proven by the intercept and slope values not statistically different from 0 and 1, respectively, in both comparisons. A slight but significant systematic negative bias was detectable between ARKRAY HA-8190V and Trinity Premier Hb9210 (mean bias = -2.0 mmol/mol).

Usability

The HA-8190V was found to be a very easy-to-use analyzer. Its main usability characteristics are reported in Table 3. Among the features which were evaluated, the possibility of switching to the Fast mode for samples previously analyzed, time savings in not having to align the barcoded tubes and the user-friendly software were particularly appreciated. In summary, the results of the usability study show that the instrument is rated to be acceptable in all cases.

DISCUSSION

In this study, we have evaluated the HA-8190V, a recently developed HbA_{1c} analyzer from ARKRAY. Our work integrates a previously published evaluation (7) without any overlap. Indeed, in the first one the ADAMS A1c HA-8190V has been compared to the previous version from the same company, i.e. to the HA-8180V system, and the Trinity analyzer has been used to verify the HbA_{1c} when Hb variants were present, which were not enrolled in this present evaluation.

Table 3
Usability characteristics of ADAMS A1c HA-8190V. The score is reported according to the average of the two evaluators, who were familiar with the other instrumentation used in the present study. Score (see text): A, optimal; B, good; C, on the average; D, poor.

Attribute	Contents	Score
Throughput	62 tests/h in the Variant (V) mode; 150 tests/h in the Fast (F) mode	B (V) A (F)
Reagent handling	Reagents placed in light aluminum bags avoiding influence of sunshine/algae growth	A
	Barcode on each reagent contains all the needed information about lot and expiry date, entered by a manual barcode reader Reagent changing can also be done during the run	A
Sample processing	Sample processing Primary capacity of 100 samples, with optional side sampler up to 200	A
	Sampling either from primary tubes with cap piercing and ID scanning for blood patient samples, or from cups for hemolysates	A
	Specific racks for whole blood, anemic samples and hemolysates, automatically recognized	A
	Stirring of the tube before the analysis and automatic barcode reading of freely placed, non-aligned barcoded samples	A
	Possibility to insert urgent samples (STAT)	A
Result processing	Warnings in case of abnormal results, variant detection, low hemoglobin	A
	On-board memory allows the storage of 900 results including chromatograms, calibration and control information	B
Calibration	Normally required only after the installation of a new column (once every 4000 tests with no pre-filter change)	B
Quality control	Alarms in case of QC fails/falls outside the given range	A
Software	Touchscreen software with pull-down menus Friendly interface allowing an intuitive scrolling through pages	A
Start-up and shut-down procedure	The start-up time (30 min) can be programmed, the shut-down is automatic	A
Maintenance	No particular daily maintenance, a 7 minutes washing procedure is needed weekly	B

The precision study performed at different HbA_{1c} levels on both blood samples and control materials revealed excellent performance. The CV values for all the different components of reproducibility, never exceeded 0.7% for results expressed either in mmol/mol or % units. This result is far below the most stringent goals for reproducibility for HbA_{1c} assay [<2.8% in SI units; <2.0% in the National Glycohemoglobin Standardization Program (NGSP) units] (8) and is in accordance with the results reported in a previous evaluation (7).

A very good agreement was found between HbA_{1c} values obtained with ARKRAY HA-8190V analyzer and other frequently used routine HPLC methods. In particular, in the comparison with both Bio-Rad D-100 and Tosoh G11 an excellent concordance was found with no statistically significant differences in the results. In the comparison between ARKRAY HA-8190V and Trinity Premier Hb9210, a slight negative bias was found, though of no clinical relevance.

Other aspects concerning the general performance of the system, such as linearity, carry-over, sample stability and trueness were not among the scopes of our work, because they have already been previously evaluated by Van der Hagen et al. (7).

In conclusion, the HA-8190V exhibits excellent reproducibility, good comparability with other routine methods currently available, high throughput and convenient usability features. These characteristics make it a reliable and suitable method for routine HbA_{1c} measurement in clinical chemistry laboratories.

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CONFLCT OF INTEREST

None.

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