Comparison of Arkray/ELITech ADAMS HA-8180V® with Bio-Rad Variant™ II Turbo2.0® and Tosoh Bioscience HLC®-723G8 for HbA1c Determination

Stéphanie Badiou,^{1,2*} Julie Guillot,¹ Nils Kuster,^{1,2} Anne-Sophie Bargnoux,^{1,2} Patricia Aguilar-Martinez,³ Elodie Boissier,³ Jean-Paul Cristol,^{1,2} and Anne-Marie Dupuy¹

¹Department of Biochemistry, Montpellier University Hospital, Montpellier, France

²UMR 204 NUTRIPASS, Université Montpellier I/II, Montpellier, France

³Department of Hematology, Montpellier University Hospital, Montpellier, France

Objective: This study aimed to evaluate a new ion-exchange HPLC system to measure hemoglobin A1c (HbA1c) in comparison to two other widely used HPLC systems. **Methods:** Analytical performance and hemoglobin variants detection was assessed on the new Arkray/ELITech ADAMS HA-8180V®, in parallel to Bio-Rad Variant™ II Turbo2.0® and Tosoh Bioscience HLC®-723G8. A method comparison and concordance for the classification of patients according to the American Diabetes Association (ADA) categories was performed using kappa test. **Results:** ADAMS HA-8180V® demonstrated excellent within-run imprecision (0%) and between-run imprecision: $\leq 1.21\%$ using blood sample and $\leq 0.94\%$ using quality controls. Method comparison of ADAMS HA-8180V® with the two other systems

yielded very high coefficient correlation ($r > 0.995$). A very good agreement (kappa value ≥ 0.81) was observed between methods for the classification of patients according to the ADA categories. In addition, all systems were able to detect the presence of most classical variants such as HbC, HbS, HbD, and HbE. **Conclusion:** The Arkray/ELITech ADAMS HA-8180V® demonstrated high analytical performance similar to previous systems such as Biorad™ Variant II Turbo 2.0® and Tosoh Bioscience HLC®-723G8. The three systems allow a high-quality HbA1c measurement and appeared interchangeable for diabetes diagnosis or for the therapeutic monitoring of patients without hemoglobin variants. J. Clin. Lab. Anal. 28:428–434, 2014. © 2014 Wiley Periodicals, Inc.

Key words: HbA1c; HPLC; analytical performance; interchangeability; diabetes

INTRODUCTION

Continuous efforts of the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry (IFCC) lead to a significant improvement in HbA1c determination with reduction of interlaboratory variability and long-term reproducibility (1–4). A consensus statement on the worldwide standardization of HbA1c defines the IFCC reference system as the only valid anchor to implement standardization of the measurement (5). However, variability between methods is still observed, especially in the presence of hemoglobin variant (6–8). Today, accuracy of methods and their potential interchangeability represent a key feature in clinical practice. Indeed, beyond the use of HbA1c levels for adaptation of diabetes therapeutic

(9, 10) appeared the use of HbA1c for diagnosis of diabetes mellitus (11), reinforcing the need for laboratories to choose an accurate and reliable method. In addition, the ability of systems to detect potential interferences such as hemoglobin variant should be taken into consideration (12, 13). In this context, we evaluated the last available

*Correspondence to: Stéphanie Badiou, Department of Biochemistry, Montpellier University Hospital, CHU Montpellier, 371 Av Doyen Gaston Giraud, F34295 Montpellier Cedex 05, Montpellier, France. E-mail: s-badiou@chu-montpellier.fr

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ion-exchange HPLC system ADAMS HA-8180V® from Arkray/ELITech.

This study reported (a) analytical performances of ADAMS HA-8180V® in comparison to two other widely used HPLC systems for measuring HbA1c; (b) a method comparison of the ADAMS HA-8180V® system versus two other systems (c) concordance between HPLC systems for the classification of patients according to the American Diabetes Association (ADA) categories (d) a description of chromatograms in presence of hemoglobin variants with each systems.

METHODS

The study was declared under the number DC-2008-417 and approved by the local ethic committee.

Arkray/ELITech ADAMS HA-8180V® was evaluated in parallel to two other systems widely recognized that are Bio-Rad Variant™II Turbo2.0® and Tosoh Bioscience automated glycohemoglobin analyzer HLC®-723G8. All the three systems using IFCC calibration and providing derived NGSP value were certified by the NGSP (5). HbA1c is calculated with the formula: %HbA1c = [HbA1c] / [Total HbA] × 100. During all the evaluation study, calibration and quality controls were used according to each manufacturer's recommendations. The three HPLC analyzers are equipped with cap-piercing and on-board hemolysis systems allowing the use of closed primary tube with whole blood.

Description of Systems

- Arkray/ELITech ADAMS HA-8180V® uses an ion exchange resin composed of hydrophilic polymer of methacrylate ester copolymer. The column is maintained at 40°C. There are three buffers and the elution is achieved in a five-step phosphate buffered gradient with increasing ionic strength. The colorimetric detection used a dual wavelength of 420 nm and 500 nm. The linear range of HbA1c extended from 3 to 20% (9–195 mmol/mol) with a runtime of 90 sec in the variant mode. There is an option to insert emergency samples (STAT). The instrument can spin tubes. Samples on EDTA, heparin, and NaF can be used for HbA1c determination, according to manufacturer's recommendations.
- Bio-Rad Variant™II Turbo 2.0® is based on a cation exchange using a silice column. Normal and abnormal hemoglobin fractions were separated using a continuum ionic gradient in a runtime of 97 sec. Two dual-piston pumps in the chromatographic station deliver a buffer solution of increasing ionic strength to the analytical cartridge and then detector. Dual-wavelength

detection is performed at 415 nm and 690 nm. Only EDTA samples were recommended by manufacturers.

- Tosoh Bioscience HLC®-723G8 uses a nonporous TSKgel G8 Variant HSi Column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components in a step gradient elution. Dual-wavelength detection is performed at 415 nm and 510 nm. Runtime is 96 sec. There is a STAT position in front of the analyzer to insert emergency samples. Samples on EDTA, heparin, citrate, and NaF can be used for HbA1c determination.

Analytical Performance Assessment

Imprecision study

Imprecision was determined using EDTA blood samples selected from the routine lab with low (<5.7%, 39 mmol/mol), medium (6–6.5%, 42–48 mmol/mol) and high (8–8.5%, 64–69 mmol/mol) levels of HbA1c. Samples were measured in 20 replicates in a same run for each level to determine within-run imprecision. Between-run imprecision was determined for three levels of HbA1c (low, medium, and high) by measuring twice a day (with a minimum interval of 4 hr between the two measures) during ten consecutive workdays. Samples were stored at 4°C during all the study, although exceeding the 7 days recommended for the Variant™II Turbo 2.0®. Additionally, between-run imprecision was calculated using low- and high-quality controls supplied by each manufacturer and measured on each analyzer once a day during 20 days.

Carryover

Carryover was assessed with samples having low HbA1c (L) (4%, 20 mmol/mol) and high HbA1c (H) (10%, 86 mmol/mol) using the sequence: H1-H2-H3-L1-L2-L3 and the formula (L1-L3)/((H1+H2+H3)/3) × 100. The same sequence was performed using A/S variant followed by a sample A/A.

Erythro sedimentation

A same sample was placed in position 1 and 10 of a rack for each analyzer. Samples with low, medium, and high levels of HbA1c were tested.

Method Comparison

Residual whole blood specimen (collected on EDTA) from 160 routine HbA1c samples with HbF <1% and without hemoglobin variant were tested on the three HPLC systems within 2 hr. We compared the

TABLE 1. Within-(A) and between- (B and C) Run Imprecision for HbA1c Determination

(A) Within-run imprecision using EDTA whole blood samples (<i>n</i> = 20)						
	HA-8180V®		VARIANT™II Turbo 2.0®		HLC®-723G8	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Low	5.30%	0	5.34%	0.94	5.29	0.34
Medium	6.10%	0	6.40%	0	6.24	0.61
High	8.30%	0	8.49%	0.34	8.37	0.33
(B) Between-run imprecision using EDTA whole blood samples (<i>n</i> = 20)						
	HA-8180V®		VARIANT™II Turbo 2.0® ^a		HLC®-723G8	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Low	5.50%	0.90	5.40%	2.70	5.50%	1.10
Medium	6.24%	1.21	6.07%	2.38	6.20%	0.86
High	8.48%	0.28	8.50%	1.68	8.56%	0.50
(C) Between-run imprecision using quality control of each system (<i>n</i> = 20)						
	HA-8180V®		VARIANT™II Turbo 2.0®		HLC®-723G8	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Level 1	5.45%	0.94	5.66%	1.07	5.75%	0.68
Level 2	9.34%	0.84	9.78%	0.85	10.58%	0.48

^aWhole blood sample was stored beyond the 7 days recommended by manufacturers.

HA-8180V® versus Variant™II Turbo 2.0® versus HLC®-723G8.

agreement for kappa value ranging from 0.61 to 0.80 and moderate agreement for kappa value between 0.41 and 0.60.

Concordance of Systems

The concordance between systems was assessed by analyzing individual classification of patients according to the three categories defined by the ADA (11) (a) normal: HbA1c < 5.7% (<39 mmol/mol); (b) at increased risk to develop diabetes: 5.7% (39 mmol/mol) to 6.4% (46 mmol/mol); (c) criterion of diabetes: HbA1c ≥ 6.5% (≥48 mmol/mol).

Hemoglobin Variants

The most common hemoglobin variants worldwide including HbA/C (*n* = 2) HbA/D (*n* = 2), HbA/E (*n* = 2), HbA/S (*n* = 6) were tested, as well as some uncommon variants such as Hope (*n* = 1), G-Philadelphia (*n* = 1), J-Baltimore (*n* = 1), O-Arab (*n* = 2), D-Punjab (*n* = 1), Winnipeg (*n* = 1), or Lepore (*n* = 1).

Statistical Analysis

Method comparison was performed using least squares linear regression analysis with determination of the slope, intercept, and coefficient of correlation. In addition, the scatter of differences was visualized on Bland-Altman plots (14). Samples outside a 7% relative bias were identified as recommended by the College of American Pathologists and the NGSP (5). Concordance between methods was assessed through Cohen's Kappa test (15). Very good agreement was defined for kappa value ≥ 0.81; good

RESULTS

Analytical Performances

Imprecision

The within-run and between-run imprecision are summarized in Table 1A and B, respectively. Within-run coefficient of variation (CV) for low, medium, and high HbA1c levels were <1% for all the three systems tested. Excellent results were obtained for ADAMS HA-8180V® as within-run imprecision was equal to 0% and between-run imprecision ≤ 1.21%, using blood samples. Between-run imprecision was even better using control quality material (Table 1C).

Carryover

The rate of carryover between samples with high and low HbA1c value or between sample with variant A/S and normal HbA was < 0.01% for the three systems.

Erythro sedimentation

Absolute differences (NGSP unit) between results of the same sample positioned in first and last position of a rack was equal to 0 for ADAMS HA-8180V®, whatever the level of HbA1c tested (of note, the analyzer is equipped with a system to spin the tubes). Absolute differences ranged from 0 to 0.1 with the Variant™II Turbo 2.0®

Fig. 1. Method comparison of ADAMS HA-8180V® versus (A) Biorad Variant™ II Turbo 2.0® and (B) HLC® 723G8.

and from 0 to 0.04 with the HLC®-723G8 for the three levels of HbA1c tested (low, medium, and high).

Method Comparison

Comparison of the ADAMS HA-8180V® with the Variant™ II Turbo 2.0® showed a strong correlation ($r = 0.996$), with a mean of difference (Variant™ II Turbo 2.0® - ADAMS HA-8180V®) of 0.08% (Fig. 1A). Only two samples among 160 were outside a 7% relative difference.

Likewise, the coefficient of correlation between the ADAMS HA-8180V® and the HLC®-723G8 was very

good (0.997). The mean of difference (HLC®-723G8-ADAMS HA-8180V®) was 0.015% (Fig. 1B). There was only one sample among 160 outside a 7% relative difference.

Concordance of Systems

The concordance of methods according to the classification of patients in the three clinical categories of ADA was presented in Table 2. A very good agreement (kappa value ≥ 0.81) was observed between the ADAMS HA-8180V® and the two other systems. Indeed, exact matches were obtained for 92.5% of patients between ADAMS

TABLE 2. Concordance between HPLC Systems for Classification of patients According to the ADA Categories

	<5.7% (<39 mmol/mol)	5.7–6.4% (39–46 mmol/mol)	≥ 6.5% (≥48 mmol/mol)
ADAMS HA-8180V ® versus VARIANT ™ II TURBO 2.0 ®			
<5.7% (<39 mmol/mol)	68	9	0
5.7–6.4% (39–46 mmol/mol)	1	37	2
≥ 6.5% (≥48 mmol/mol)	0	0	43
ADAMS HA-8180V ® versus HLC ® -723G8			
<5.7% (<39 mmol/mol)	76	1	0
5.7–6.4% (39–46 mmol/mol)	4	36	0
≥ 6.5% (≥48 mmol/mol)	0	2	41

Kappa test: 0.884: Very good agreement.

Kappa test: 0.931: Very good agreement.

HA-8180V® and Variant™ II Turbo 2.0® and for 95.6% of patients between ADAMS HA-8180V® and HLC®-723G8.

Hemoglobin Variants

Detection of common hemoglobin variants was observed for each system, leading to an abnormal profile on the chromatograms. ADAMS HA-8180V® likewise the two other systems can detect and identify HbS and HbC variants as additional fractions named S or C and excluded from the calculation of HbA total area (Fig. 2). The Variant™ II Turbo2.0® indicated HbC in a “C window”, while the other variants (S, D, and E) appeared in a “variant window”. On the HLC®-723G8, Hb S was eluted in a separate peak after the A0 fraction, named “HV-1”, the HbC in a peak “HV-2” and the HbD in a peak “HV-0”. A recent update of HLC®-723G8 software was performed to improve the detection of HbE in an “HV-3” fraction. On ADAMS HA-8180V®, the presence of HbD or HbE led to a comment “abnormal fraction” without available value of HbA1c.

The rare variants, Hb Hope, G-Philadelphia, J-Baltimore, O-Arab, D-Punjab, Lepore, and Winnipeg led to the presence of an abnormal peak on the chromatograms for all systems.

DISCUSSION

This study demonstrated very high analytical performance of the new ADAMS HA-8180V® analyzer, comparable to performance of Variant™ II Turbo2.0® and HLC®-723G8.

Using, either blood samples and quality controls, between-run CV with ADAMS HA-8180V® was <2% as recommended by the National Academy of Clinical Biochemistry (16). Our study highlighted high correlation and concordance between the new ADAMS HA-8180V® and the two other widely used methods, Bio-Rad

Variant™ II Turbo 2.0® and Tosoh Bioscience HLC®-723G8 (17, 18).

Due to the continuous efforts of the IFCC, a worldwide standardization of HbA1c measurement is committed (5). This step represents an important feature in clinical management of diabetic patients. A few years ago, it was recommended to use the same methods for the follow-up of patients. Today it is recommended that laboratories only use HbA_{1c} assay methods that are certified by the NGSP as traceable to the Diabetes Control and Complications Trial reference. In addition, manufacturers should also show traceability to the IFCC reference method (16). One benefit of the standardization is the possibility to have interchangeable methods without affecting the results of HbA1c.

As illustrated in the present study, clinical assessment of patients in the three categories defined by the ADA (11): <5.7% (<39 mmol/mol), 5.7–6.4% (39–46 mmol/mol), and ≥6.5% (≥48 mmol/mol), provided a very good agreement between the ADAMS HA-8180V® and the two other methods. These results demonstrated the potential interchangeability between methods in clinical assessment of patients.

Beyond their high analytical performance, the three HPLC systems revealed an effective detection of the presence of hemoglobin variants. An exploitable result was obtained for the three systems in case of heterozygous A/S or A/C patients, in agreement with recent studies (8, 19). Depending on methods, caution should be taken when interpreting HbA1c in case of A/D and A/E variants (20). The present study was not designed to evaluate the accuracy of results in case of hemoglobin variant because only few samples were tested, without reference value. To ensure a better detection of variant, it should be mandatory to perform a visual reading of chromatograms by technicians instead of an automated validation through dedicated software or a middleware. Although samples with Hb variant are often in the normal range, very low (<4%, 20 mmol/mol) or very high (>40%, 414 mmol/mol) values of HbA1c should alert to the

A Hb A/S

B HbA/C

Fig. 2. Chromatograms with each system for common HbS (2A) and HbC (2B) variants.

potential presence of an Hb variant. In addition, HbA1c values that were not consistent with history of patients should be further investigated (16). Presence of hemoglobin variant must be indicated when reporting HbA1c results due to potential interference leading to a misclassification of patients. In these cases, patients should be considered as their own control for their monitoring and followed on the same system.

In conclusion, this study highlighted a high analytical performance of the new Arkray/ELITech ADAMS HA-8180V® comparable to the previous Biorad™ Variant II Turbo 2.0® and Tosoh Bioscience HLC®-723G8 allowing an accurate HbA1c measure. With the benefit of the IFCC standardization, the three HPLC systems appeared totally interchangeable for the diagnosis of diabetes or for the therapeutic monitoring in patients without hemoglobin variants.

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