Practical Laboratory Medicine 5 (2016) 57–64

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Evaluation of Bio-Rad D-100 HbA1c analyzer against Tosoh G8 and Menarini HA-8180V

José María Maesa, Patricia Fernández-Riejos*, Catalina Sánchez Mora, María de Toro, Paloma Menéndez Valladares, Concepción González-Rodriguez

Clinic Biochemistry Department, Virgen Macarena University Hospital, Spain

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form
4 May 2016
Accepted 11 May 2016
Available online 14 May 2016

Keywords:
Glycated hemoglobin
High performance liquid chromatography
Analyzer
Intra-assay variability
Inter-assay variability

ABSTRACT

Objectives: To evaluate the Bio-Rad D-100®, an HPLC analyzer for glycated hemoglobin (HbA_{1c}) determination, and to compare its performance with the Menarini HA-8180V® and Sysmex G8®.

Methods: Method comparison was performed according to Clinical and Laboratory Standards Institute (CLSI) EP9-A2 guidelines. We selected 100 samples from the routine laboratory workload and analyzed them in duplicate with the three analyzers. The imprecision study was performed according to CLSI EP5-A2 guidelines for both inter-assay and intra-assay variability. Bias was assessed with external quality control material. To establish linearity, CLSI EP6-A protocol was followed.

Results: Method comparison (95% confidence intervals in parentheses): D-100 vs G8: Passing-Bablok regression; $y=0.973(0.963–0.983)\times-0.07(-0.07-0.069)$; $r=0.9989$. Bland-Altman mean difference: $-0.229\%HbA_{1c}$ (-0.256: -0.202); Relative bias plot: D-100/G8 vs D100-G8 mean ratio$=0.971(0.967-0.975)$. D-100 vs HA-8180V: Passing-Bablok regression; $y=0.944(0.932–0.958)\times-0.078(0.024-0.173)$; $r=0.9989$. Bland-Altman mean difference: $-0.363\%HbA_{1c}$ (-0.401: -0.325); Relative bias plot D-100/HA-8180V vs D100-HA-8180V mean ratio$=0.955(0.952-0.958)$. Inter-assay coefficient of variation (CV): 0.81%. Intra-assay CV: 1.04% (low level), and 0.78% (high level). Bias against target value$=2.332\%$. Linearity: $r^2=0.998$ in the concentration range $4.4-13.9\%HbA_{1c}$. Carry-over: 0.0024%.

Conclusions: The Bio-Rad D-100 shows good correlation with G8 and HA-8180V. There is a small proportional systematic difference (2.7% and 5.6%, respectively) in both comparisons. Inter and intra-assay CVs are both lower than the lowest CV obtained in studies performed with D-100 and other instruments.

1. Introduction

Diabetes Mellitus (DM) has become a global epidemic whose incidence is increasing and which already affects one in twelve people [1] and reduces the quality of life for many millions of people through its complications (retinopathy, renal, vascular and heart diseases, etc.). As a result, glycated hemoglobin (HbA_{1c}) is a fundamental parameter in the clinical laboratory, as it has been determined to be an effective, stable and comfortable alternative to blood glucose for both

* Corresponding author.
E-mail address: patricia.fernandez.sspa@juntadeandalucia.es (P. Fernández-Riejos).

http://dx.doi.org/10.1016/j.plabm.2016.05.002

diagnosis and monitoring DM [2,3].

HbA_{1c} is an ideal parameter for diagnosis and control of DM because there is a direct relationship between average blood glucose levels and HbA_{1c} concentration [4]. HbA_{1c} is a derivative of hemoglobin, a minor fraction that is generated as a result of non-enzymatic reaction between the aldehyde group of the open structure of the glucose molecule and the free amino group in the terminal valine of the hemoglobin β chain. This reaction occurs with the formation of an unstable intermediate, a Schiff base known as labile hemoglobin. Then, after an Amadori rearrangement, it is stabilized in its ketoamine form constituting HbA_{1c} [5].

HbA_{1c} can be determined by different techniques [6], which are based on chemical and structural differences between HbA_{1c} and other hemoglobin fractions. One of those techniques is ion exchange High Performance Liquid Chromatography (HPLC), which exploits the fact that the ionization point (pI) of HbA_{1c} is different from other hemoglobin fractions. It uses a cation exchange resin to separate HbA_{1c}.

Although HbA_{1c} determination for diagnosing and monitoring DM has some limitations, such as the presence of some possible interferences (hemoglobinopathies, carbamylated and labile hemoglobin) or altered half-life of red blood cells (renal failure, hemolytic anemia, etc.), the advantages are many [7]. In addition, many of the disadvantages have been overcome in the newly developed analyzers and for many of them the most common hemoglobin variants do not pose a problem, nor do the carbamylated hemoglobin [8] or labile hemoglobin [6].

HPLC techniques are considered the gold standard in the determination of HbA_{1c} and are among the most widespread in clinical laboratories. In this study we compared the performance of three HPLC analyzers for HbA_{1c}.

2. Materials and methods

We studied three HbA_{1c} analyzers: G8® from Tosoh Bioscience (Tokyo, Japan) (G8); D-100® from Bio-Rad Laboratories (Hercules, CA, USA) (D-100); and HA-8180V® from A Menarini Diagnostics (Firenze, Italy) (HA-8180V). These three instruments employ the same measurement principle: ion exchange HPLC, preceded by a pre-filter to avoid the rapid deterioration of the column. The detection system is a photometer, which quantifies the different hemoglobin fractions identified by their specific retention time.

The instruments were calibrated according to the manufacturers' specifications and we performed daily internal quality control with two levels of quality control, one high and one low, provided by the manufacturers.

3. Assessment protocol

3.1. Method comparison

Method comparison was performed according to Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA) EP9-A2 guidelines. From venous blood samples (collected into EDTA tubes) received for the determination of HbA_{1c} in the routine laboratory, we selected 100 samples and divided them into 4 concentration ranges (25 samples per range): 4–6% HbA_{1c} (20.22–42.08 mmol/mol Hb), 6–8% HbA_{1c} (42.08–63.93 mmol/mol Hb), 8–10% HbA_{1c} (63.93 − 85.79 mmol/mol Hb) and $>10\%$ HbA_{1c} (>85.79 mmol/mol Hb), overall concentration range of the samples used: 4.6–12.15% HbA_{1c} (26.78–109.29 mmol/mol Hb). We analyzed the samples in duplicate on all three analyzers with less than 2 h between the first and last samples.

3.2. Precision

The precision study was performed according to CLSI EP5-A2 guidelines for both inter-assay and intra-assay variability.

The inter-assay variability was determined with a pool of samples, aliquoted and frozen at −80 °C. We measured one aliquot per day for twenty consecutive days.

For intra-assay variability we selected two samples: one with a high HbA_{1c} value and other with a low HbA_{1c} value. We measured both samples twenty consecutive times in the same run.

3.3. Accuracy

The bias of the method was assessed by analyzing for twenty consecutive days the three levels of Liquicheck® Diabetes Control from Bio-Rad (Hercules, CA), prepared from whole human blood for external quality control. Using the results obtained from the D-100 and the target value we calculated total and relative bias.

3.4. Linearity

To establish the linearity, CLSI EP6-A protocol was followed. We selected two samples with values 4.4% and 13.9% $HbA_{1c,}$ respectively (both samples had the same hemoglobin concentration, 13.7 g/dL). The samples were mixed according to the

proportions 0:4, 1:3, 2:2, 3:1, 4:0, (v/v), and HbA_{1c} content was determined for each mixture.

3.5. Carry-over

Carry-over was assessed following the "Protocols for Determination of Limits of Detection and Limits of Quantitation" (1986) [9]. We used 2 samples: High sample (H) with 14% HbA_{1c}, and Low sample (L) with 5% HbA_{1c}. We analyzed them in the following order: L1L2L3H1H2L4H3H4L5L6L7L8H5H6L9H7H8L10H9H10L11 [9].

3.6. Statistical analysis

For the method comparison we used Passing-Bablok non parametric regression and Pearson correlation coefficient (r) was calculated. The confidence intervals (95%) were determined. The bias was estimated using a Bland-Altman plot and a relative difference plot. Coefficients of variation (CV%) were calculated to estimate the intra and inter-assay variability. Data analysis and statistical calculations were performed using Method Validator (Philippe Marquis Software) and SPSS (IBM Analytics, Armonk, NY, USA).

4. Results

4.1. Method comparison

- D-100 vs G8

Passing-Bablok non parametric regression analysis showed a slope of 0.973 with a 95% confidence interval (CI) of (0.963–0.983), and an intercept of −0.07, with CI (−0.07: −0.069) (Fig. 1). The Pearson Correlation Coefficient between both measurement methods was 0.9989.

The Bland-Altman plot (Fig. 2) shows a mean difference of −0.229%HbA_{1c} (CI: −0.256: −0.202).

Relative bias plot (Fig. 3) D-100/G8 vs D100-G8 gives a mean ratio (D-100/G8) of 0.971 (CI: 0.967−0.975).

- D-100 vs HA-8180V

Passing-Bablok regression (Fig. 4) showed a slope of 0.944 (CI: 0.932–0.958), and intercept of 0.078 (CI: −0.024−0.173); r=0.9989 (Fig. 3).

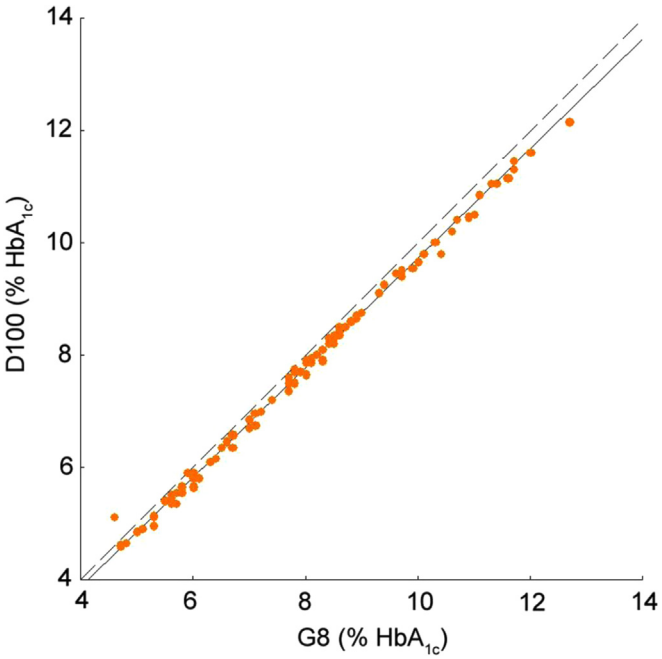

Fig. 1. Passing-Bablok non-parametric regression; D-100 vs G8; y=0.973(0.963–0.983) × −0.07(−0.07−0.069); r=0.9989. (95% confidence intervals in parentheses).

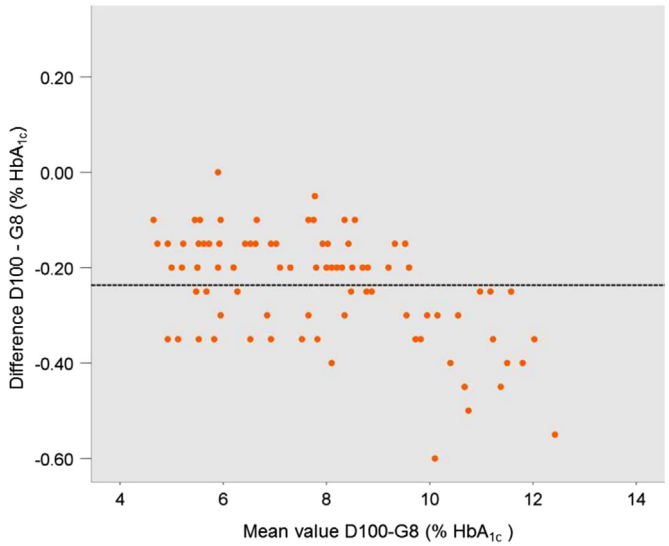

Fig. 2. Bland-Altman difference plot; D-100 vs G8; mean difference (D-100 – G8) = – 0.229% HbA_{1c} (95% CI: – 0.256: – 0.202).

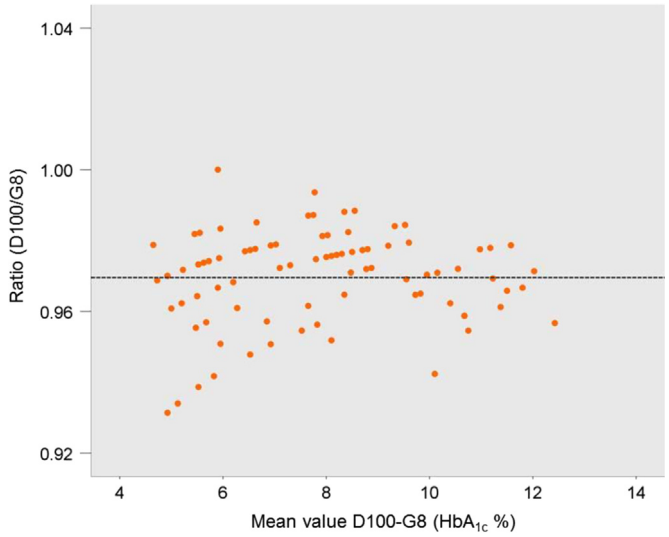

Fig. 3. Relative bias plot; D-100/G8 vs mean value of D100 and G8; mean ratio (D-100/G8) = 0.971 (95% CI: 0.967 – 0.975).

The Bland-Altman plot (Fig. 5) gives a mean difference of – 0.363%HbA_{1c} (CI: – 0.401: – 0.325).

Relative bias plot (Fig. 6) D-100/HA-8180V vs mean of D100 and HA-8180V gives a mean ratio (D-100/HA-8180V) of 0.955(0.952 – 0.958). Fig. 7.

4.2. Imprecision and bias

Results for inter-assay and intra-assay variability are shown in Table 1 in both National Glycohemoglobin Standardization Program (NGSP; % HbA_{1c}) and International Federation of Clinical Chemistry (IFCC; mmol/mol Hb) units.

4.3. Linearity and carry-over

The instrument response in the concentration range between 4.4% and 13.9% HbA_{1c} (24.6–128.4 mmol/mol Hb) was completely linear, ($r^2 = 0.9935$). The percentage of carry-over calculated for the instrument was 0.0024% in NGSP units (%HbA_{1c}) and 0.026% in IFCC units (mmol/mol Hb).

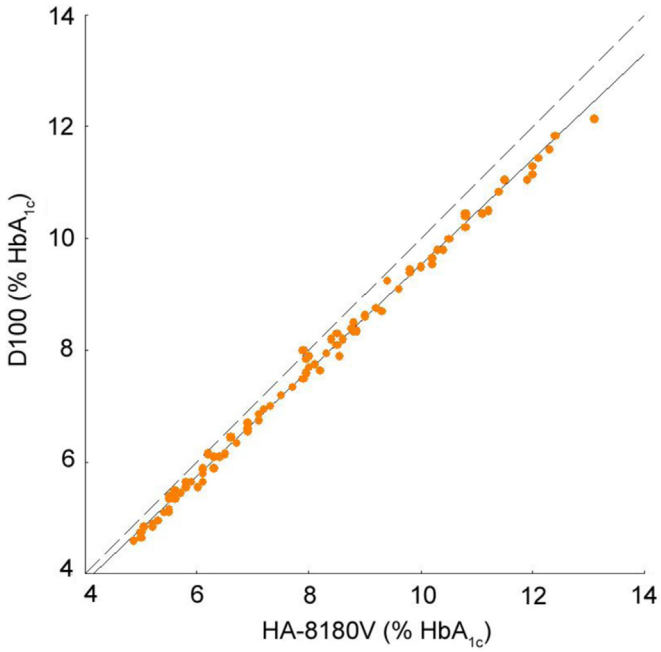

Fig. 4. Passing-Bablok non-parametric regression; D-100 vs HA-8180V; y=0.944(0.932–0.958) × −0.078 (0.024−0.173); r=0.9989. 95% confidence intervals in parentheses.

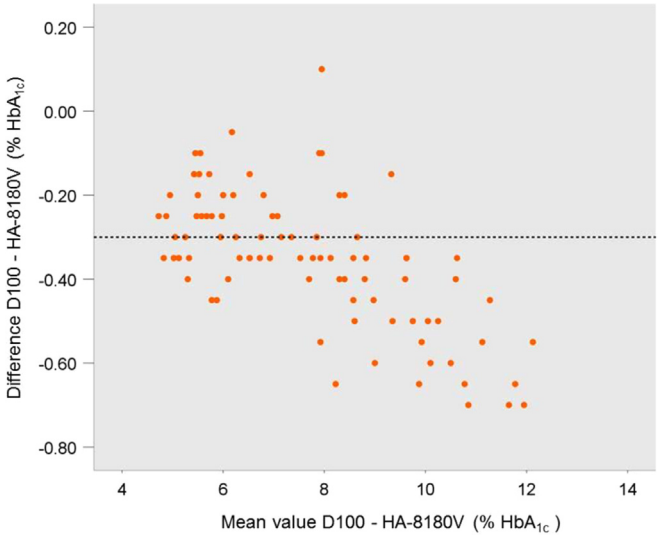

Fig. 5. Bland-Altman difference plot; D-100 vs HA-8180V; mean difference (D-100−HA-8180V)= −0.363 (95% CI: −0.401: −0.325).

5. Discussion

The Bio-Rad D-100 shows good correlation with both reference instruments Tosoh G8 and Menarini HA-8180V, with a Pearson correlation coefficient of 0.998, the same for both comparisons. This value agrees with the one published in another similar study, in which the D-100 is compared with another HPLC instrument [10].

On a more detailed analysis with a Passing-Bablok regression, it was observed that, both using G8 as reference instrument, and in the comparison with HA-8180V, the intercepts for both regression lines did not show statistically significant differences between the methods. The 95% confidence intervals (CI) both included zero. However, the slopes, with confidence intervals (0.963–0.983) and (0.932–0.958), for G8 and HA-8180V respectively, slightly deviate from the ideal slope, showing a small proportional systematic difference that can be quantified at 2.7% against the G8 and 5.6% against the HA-8180V.

This systematic difference appears again in the results in Bland-Altman plots (Figs. 2 and 5), which shows a negative bias for high HbA_{1c} values present in both comparisons, quantified in the mean differences obtained. In the comparison with G8

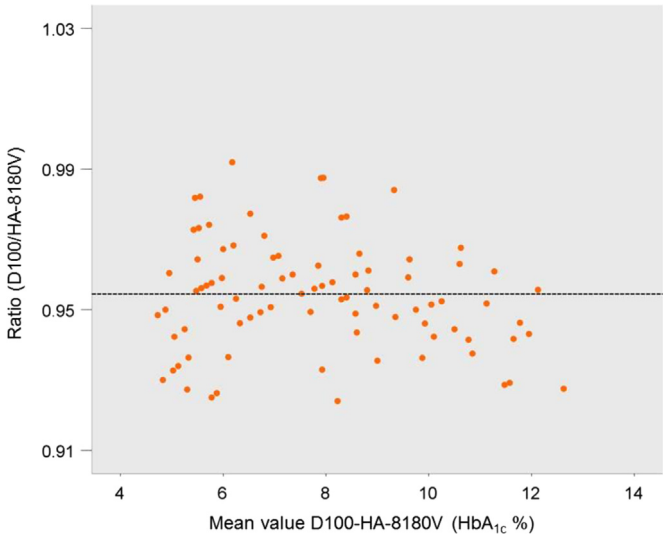

Fig. 6. Relative bias plot; D-100/HA-8180V vs mean value of D-100 and HA-8180V; mean ratio (D-100/HA-8180V)=0.955 (95%CI: 0.952 – 0.958).

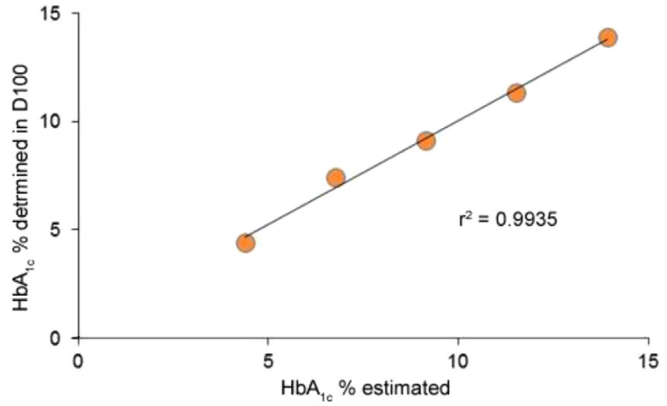

Fig. 7. Linearity of the instrument in the range between 4.4% and 13.9% HbA_{1c} (24.6–128.4 mmol/mol Hb). In the graph we plotted the values obtained in D-100 for the 5 dilutions on the Y axis, versus the theoretical values calculated for the dilutions on the X axis.

was −0.23%HbA_{1c}, (CI: −0.202; −0.256); and with HA-8180V was −0.36%HbA_{1c}, (CI-0.401; −0.325). When we evaluated the differences with a relative bias plot (Figs. 3 and 6), there is still an important bias present, but it affects the whole range of HbA_{1c} concentrations, reaching in the case of D100/G8 plot a similar value to the proportional error obtained for the Passing-Bablok: −2.9% (mean ratio=0.971) but higher in D-100/HA-8180V plot: −4.5% (mean ratio=0.955).

This problem with bias also appears in the accuracy study. The evaluation of bias using an external quality control (Table 1) proved again the existence of a negative bias in D-100. And, as the results in Bland-Altman plot expressed, the bias is greater for higher HbA_{1c} concentrations (1.55% for Liquicheck 1 at 5.34%HbA_{1c}, 1.931% for Liquicheck 2 at 9.5% HbA_{1c}, and 3.515% for Liquicheck 3 at 14.6%HbA_{1c}). This progressive bias might be due to a problem with the calibration protocol or traceability of calibration materials. Nevertheless, it seems to affect mainly higher HbA_{1c} concentrations, in which case it would not be a critical interference in clinical or diagnostic decisions.

HbA_{1c} is a longitudinal parameter, used in monitoring diabetes over the patient's lifetime. Therefore, the reproducibility and repeatability of HbA_{1c} measurements must be well controlled. To assess the variability in the D-100 instrument, we determined intra-assay CV with two samples, one with a low concentration of HbA_{1c} (4.7%) and one with a high concentration (11.4%), obtaining CV values of 1.05% (%HbA_{1c}) [1.97% (mmol/mol)] and 0.78% (%HbA1c) [0.99% (mmol/mol)], respectively. Both CVs are lower than the lowest CV obtained in several studies performed with the D-100 [10] and with other instruments [11]. Also, these CV values meet the recommendations made by Sacks et al. [12] of within-laboratory CV below 2%. The inter-assay CV% estimated was 0.81% (%HbA_{1c}) [1.51% (mmol/mol)], an excellent result which meets the criteria required for the determination of HbA_{1c} [13], which are higher in IFCC units (mmol/mol) than in NGSP units (% HbA_{1c}) [14].

The linear response of the instrument has been confirmed in the range between 3.4% and 13.9%HbA_{1c} (24.6–128.4 mmol/mol), which represents the HbA_{1c} concentration range involved in diagnostic decisions.

Table 1.
Results of the imprecision and bias study in both %HbA_{1c} and mmol/mol Hb units. The inter-assay variability was calculated measuring the same pool for twenty consecutive days. For intra-assay variability two samples: one high and one low HbA_{1c} concentration were processed twenty consecutive times. To estimate bias we used the target values for three levels of an external quality control material (Liquichek Diabetes Control®; Bio-Rad).

Precision (%HbA_{1c})		Mean	SD	CV (%)	
Inter-assay variability		6.06	0.049	0.81	
Intra-assay variability		11.39	0.089	0.78	
		4.75	0.050	1.05	
Bias (%HbA_{1c})	Level 1	Level 2	Level 3		Average
	(5.34)	(9.5)	(14.6)		
Total	−0.082	−0.18	−0.496		−0.252
%	1.55	1.931	3.515		2.332
Precision (mmol/mol Hb)		Mean	SD	CV (%)	
Inter-assay variability		42.46	0.641	1.51	
Intra-assay variability		100.98	0.997	0.99	
		28.36	0.558	1.97	
Bias (mmol/mol Hb)	Level 1	Level 2	Level 3		Average
	(34.8)	(80.4)	(136)		
Total	−0.827	−2.039	−5.352		−2.739
%	2.435	2.602	4.097		3.045

In some instruments carry-over can distort the results of samples following a sample with high concentration of the analyte. Carryover for the D-100 was tested using the method described by Peters [10]. The result is a carry-over quantified as 0.0024% (%HbA_{1c})/0.026% (mmol/mol Hb), which is very satisfactory and similar to carry-over calculated for other analyzers [15] ensuring that there is no contamination after measuring concentrated samples.

Finally, D-100 provides a number of advantages in daily operation, such as the incorporation of computer and control software on the instrument itself, controlled through an easy-to-use touch screen. Through the screen the operator has access to all system controls and configurations; maintenance procedures, change of reagents, performance of analyses, data collection and inspection of chromatograms. The change of reagents, column and pre-filter is very simple and can be done with the equipment running, it not being necessary to change the column for up to 10,000 determinations. Also an excellent throughput should be noted allowing the achievement of only 45 s per determination, which is better than the throughput of the G8 and HA-8180V.

References

[1] F. Aguiree, A. Brown, N.H. Cho, G. Dahlquist, S. Dodd, T. Dunning, et al., IDF Diabetes Atlas, sixth edition, . International Diabetes Federation, Basel, 2013.
[2] World Health Organization, Use of Glycated Haemoglobin (HbA1c) in Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation, Geneva, World Health Organization, 2011, pp. 1–25. Available from: ⟨http://www.who.int/diabetes/publications/diagnosis_diabetes2011/en/⟩ (accessed May 2016).
[3] The International Expert Committee, International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes, Diabetes Care 32 (7) (2009) 1327–1334, http://dx.doi.org/10.2337/dc09–9033. (accessed May 2016).
[4] K.H. Gabbay, K. Hasty, J.L. Breslow, R.C. Ellison, H.F. Bunn, P.M. Gallop, Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus, J. Clin. Endocrinol. Metab. 44 (5) (1977) 859–864.
[5] S.M. Osterman-Golkar, H.W. Vesper, Assessment of the relationship between glucose and A1c using kinetic modeling, J. Diabetes Complicat. 20 (5) (2006) 285–294 ⟨http://www.ncbi.nlm.nih.gov/pubmed/16949515⟩. (accessed May 2016).
[6] C. Weykamp, W.G. John, A. Mosca, A review of the challenge in measuring hemoglobin A1c, J. Diabetes Sci. Technol. 3 (3) (2009) 439–445.
[7] A. Lapolla, A. Mosca, D. Fedele, The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: still a long way to go, Nutr. Metab. Cardiovasc Dis. 21 (7) (2011) 467–475, http://dx.doi.org/10.1016/j.numecd.2011.02.006. (accessed May 2016).
[8] R.C. Dolscheid-Pommerich, S. Kirchner, C. Weigel, L. Eichhorn, R. Conrad, B. Stoffel-Wagner, et al., Impact of carbamylation on three different methods, HPLC, capillary electrophoresis and TINIA of measuring HbA1c levels in patients with kidney disease, Diabetes Res. Clin. Pract. 108 (1) (2015) 15–22 ⟨http://linkinghub.elsevier.com/retrieve/pii/S0168822715000546⟩. (accessed May 2016).
[9] T. Peters, J.O. Westgard, Evaluation of methods: protocols for determination of limits of detection and limits of quantitation, in: N.W. Tietz (ed), Textbook of Clinical Chemistry, 3rd edn., Philadelphia, WB Saunders, 1986, p. 421.
[10] S. Jaisson, N. Leroy, E. Guillard, A. Desmons, P. Gillery, Analytical performances of the d-100TM hemoglobin testing system (Bio-Rad) for HbA1c assay, Clin. Chem. Lab. Med. 53 (9) (2015) 1473–1479 ⟨http://www.degruyter.com/view/j/cclm.ahead-of-print/cclm-2015–0288/cclm-2015–0288.xml⟩. accessed May 2016.
[11] F. Ramon, M.J. Alsina, B. Boned, C. Biosca, S. Bullich, F. Cava, et al., XVII Programa de Garantía Externa de la Calidad de Bioquímica (Hemoglobina A1c) de la Sociedad Española de Bioquímica Clínica y Patología Molecular, 2013, pp. 199–203.
[12] D.B. Sacks, M. Arnold, G.L. Bakris, D.E. Bruns, A.R. Horvath, M.S. Kirkman, et al., Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus, Clin. Chem. 57 (6) (2011) e1–47, http://dx.doi.org/10.1373/clinchem.2010.161596. (accessed May 2016).
[13] P.H. Petersen, Performance criteria based on true and false classification and clinical outcomes. Influence of analytical performance on diagnostic outcome using a single clinical component, Clin. Chem. Lab. Med 53 (6) (2015)

849–855 ⟨http://www.degruyter.com/view/j/cclm.2015.53.issue-6/cclm-2014–1138/cclm-2014–1138.xml⟩. (accessed May 2016).
[14] C.W. Weykamp, A. Mosca, P. Gillery, M. Panteghini, The analytical goals for hemoglobin A1c measurement in IFCC units and National Glycohemoglobin Standardization Program units are different, Clin. Chem. 57 (2011) 1204–1206.
[15] C. Weykamp, E. Lenters-Westra, H. van der Vuurst, R. Slingerland, C. Siebelder, W. Visser-Dekkers, Evaluation of the Menarini/ARKRAY ADAMS A1c HA-8180V analyser for HbA1c, Clin. Chem. Lab. Med. 49 (4) (2011) 647–651 ⟨http://www.ncbi.nlm.nih.gov/pubmed/21323625⟩. (accessed May 2016).