ORIGINAL ARTICLE

Evaluation of 3 Hemoglobin A_1c Point of Care Instruments. Point of Care Testing for HbA_1c: Evaluation of Cobas b101, B-Analyst and Afinion™

María de Toro-Crespo, Catalina Sánchez-Mora, Patricia Fernández-Riejos, José M. Maesa-Márquez, Concepción González-Rodríguez

Clinic Biochemistry Department, Virgin Macarena University Hospital, Spain

SUMMARY

Background: **The aim of our study was to evaluate three POC instruments for the measurement of glycated hemoglobin (HbA_1c) (Cobas b101 (Roche Diagnostics®), Afinion ™ (Alere Technologies), and B-analyst (Menarini Diagnostics)), which were compared to G8 (Tosoh®) as the reference method. In addition, the inter-assay and intra-assay variability, and linearity of the different techniques were analyzed.**

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, as well as with the reference method. The imprecision study was performed according to CLSI EP5-A2 guidelines for both inter-assay and intra-assay variability. The inter-assay variability was estimated from aliquots of a sample obtained from a blood pool with an HbA_1c value of 6.1% as determined by the reference method. To establish linearity, the CLSI EP6-A protocol was followed.**

Results: **Method comparison (95% confidence intervals in parentheses): Passing-Bablok regression between the Cobas b101 and the G8, the slope was 0.886 (0.865, 0.909), y-intercept: 0.80 (0.61, 0.96), r = 0.99 (p < 0.05). Bland-Altman mean difference: -0.0985 (-0.0171, -0.0264). In the case of the Afinion, slope 0.967 (0.938, 1.000), y-intercept 0.263 (0.000, 0.475), r = 0.984, Bland-Altman mean difference: 0.0178 (-0.0561, 0.0917) and finally in the case of B-analyst compared to the G8 the slope: 1.036 (1.000, 1.056), y-intercept: -0.14 (-0.30, 0.10), r = 0.996 Bland-Altman mean difference: 0.124 (0.0851, 0.162). The values for CV% obtained for Cobas b101, Afinion, and B-Analyst were, respectively, for inter-assay coefficient of variation (CV): 1.92%, 2.13%, 1.34%, for intra-assay CV: 2.06%, 1.13%, 1.79% (low level), and 1.87%, 1.97%, 3.17% (high level). The three methods studied are linear in the test interval.**

Conclusions: **The results of this study show that the Cobas b101, Afinion, and B-analyst instruments present a good correlation with the reference method. In summary, the three POC HbA_1c devices assessed offer the advantages of fast and reliable test results that make their use possible to improve the care of diabetic patients as well as the possibility of establishing early treatment because of their immediate availability. They are therefore considered suitable for the control, but not for the diagnosis of diabetes.**

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Correspondence:
María de Toro-Crespo
Clinic Biochemistry Department
Virgin Macarena University Hospital
Avda Doctor Fedriani, 3
41009 Sevilla
España
Phone: +34 667737451
Email: detorocrespo.maria9@gmail.com

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INTRODUCTION

Diabetes mellitus, especially type 2 diabetes (DM2), is a chronic disease that represents an important public health problem, not only because of its high prevalence and incidence, but also because it is associated whith high morbidity and mortality rates [1].

Since 1977, HbA_1c (glycated hemoglobin) has been a useful tool for metabolic control in patients with diabetes mellitus [2] as it makes it possible to assess the risk of developing micro- and macro-complications associated with the disease [3]. In January 2014, the American Diabetes Association (ADA) updated its criteria and included an HbA_1c value $\geq 6.5\%$ (48 mmol/mol) [4] for the diagnosis of diabetes, the HbA_1c value makes it possible to estimate the probability of developing complications, as well as the need for therapeutic changes.

Different programs, such as the National Glycohemoglobin Standardization Program (NGSP), the HbA_1c harmonization program, and the IFCC Working group for standardization of HbA_1c, have made it possible to reach a consensus on the development of well-defined reference measurement procedures as well as primary reference materials [5].

In September 2007, the ADA, the International Diabetes Federation (IDF), the European Association for the Study of Diabetes (EASD), and the International Federation of Clinical Chemistry (IFCC) [6] published a Consensus Document which included different points about general standardization and the issue of analytical results for HbA_1c. Among other measures, it was decided to use the method suggested by the IFCC to calibrate different techniques for the determination of HbA_1c, as well as deliver the results in traceable units to the Diabetes Control and Complications Trial (DCCT) (National Glycohemoglobin Standardization Program [NGSP], %) and in IFCC units (mmol/mol).

Various techniques have been developed for point of care instruments (POC). The advantages offered by them are obvious and include simplification of the administrative procedures and hospital circuits, the rapid availability of results, and the simple management of the measuring instrument enabling use by professionals not experienced in laboratory techniques.

In addition since 2006, the Standards of Medical Care in Diabetes (ADA) indicate that the measurement of HbA_1c using POC analyzers provides the opportunity to make suitable treatment changes during the visit itself [4,5,7,8].

The performance of NGSP certified POC instruments, in comparison to the methods used in a conventional laboratory, have been cause of concern. Over the last few years different assessments have been made of POC instruments for determining HbA_1c and this has shown an improvement in units that did not previously meet quality specifications [9-11].

The purpose of our study is to assess three POC analyzers for measuring HbA_1c; Cobas b101 (Roche Diagnostics®), Afinion™ (Alere Technologies), and B-analyst (Menarini Diagnostics).

We have compared the results obtained with POC analyzers with our laboratory HPLC reference method, G8 (Tosoh®) that uses the validated method of cation exchange high performance liquid chromatography (HPLC). Furthermore, an analysis was made of the inter-assay and intra-assay variability and the linearity of the different techniques.

MATERIALS AND METHODS

This study used three POC HbA_1c analyzers: Cobas b101 (Roche Diagnostics), Afinion™ (Alere Technologies), and B-analyst (Menarini Diagnostics) which were compared to G8 (Tosoh®) as the reference method.

The Cobas b101 (Roche Diagnostics) uses the latex agglutination inhibition immunoassay technique with results available in 5 minutes, Afinion™ (Alere Technologies) is based on separation by affinity to boronate and the results are obtained in 3 minutes, and B-analyst (Menarini Diagnostics) is based on turbidimetric latex agglutination inhibition immunoassay with results available in 8 minutes. The reference instrument is a G8 unit (Tosoh®) that uses HPLC columns with cation exchange resin.

All the methods are standardized as per the IFCC reference procedure. The instruments were calibrated according to the manufacturers' specifications and subject to daily control with two, high and low, quality control levels provided by the manufacturers.

The analytical results were compared as described in protocol EP9-A2 CLSI [12]. The samples used were obtained from the routine Biochemistry Laboratory of Virgin Macarena University Hospital and four concentration ranges were selected: 4 - 6% HbA_1c, 6 - 8% HbA_1c, 8 - 10% HbA_1c y > 10% HbA_1c. All these samples were analyzed by the units being assessed and by the reference unit.

Variability

The intra- and inter-assay variability was determined as described in protocol CLSI EP5-A2 [13]. The inter-assay variability was estimated from aliquots of a sample obtained from a blood pool with an HbA_1c value of 6.1% as determined by the reference method and frozen to -80°C for up to one month. Measurements were made in the analizers being assessed over 20 consecutive days. Afinion only accepts samples that are not hemolyzed and have not been frozen so in this case the inter-assay variability was determined from the quality control data provided by the manufacturer, with a target value of 6.2% HbA_1c.

The intra-assay variability was estimated from two patient samples, high and low HbA_1c values (12% and 4.4%, respectively) that were processed twenty consecutive times using the same work routine on the same day.

Table 1. Passing-Bablok regression of the data obtained with Cobas b101, Afinion and B- analyst compared to G8.

	Slope	95% confidence interval	Y-intercept	95% confidence interval	r
Cobas b101	0.886	0.865, 0.909	0.80	0.61, 0.96	0.990
Afinion	0.967	0.938, 1.000	0.263	0.000, 0.475	0.984
B-analyst	1.036	1.000, 1.056	-0.14	-0.30, -0.10	0.996

Table 2. Bland-Altman plot of the data obtained with Cobas b101, Afinion, and B- analyst compared to G8.

	Mean difference	Confidence interval (95%)
Cobas b101	-0.0985	-0.0171, -0.0264
Afinion	0.0178	-0.0561, 0.0917
B-analyst	0.124	0.0851, 0.162

Table 3. Intra- and inter-assay variability of the data obtained with Cobas b101, Afinion and B-analyst.

	Intra-assay variability						Inter-assay variability		
	Mean value HbA$_1$c %	Standard deviation	CV%	Mean value HbA$_1$c	Standard deviation	CV%	Mean value HbA$_1$c %	Standard deviation	CV%
Cobas b101	11.12	0.21	1.92	5.51	0.11	2.06	6.23	0.11	1.87
Afinion	14.23	0.30	2.13	4.72	0.05	1.13	6.42	0.12	1.97
B-analyst	12.01	0.16	1.34	4.49	0.08	1.79	5.84	0.18	3.17

Linearity

The linearity was determined by making duplicate assays of a mixture of two samples with the same amount of hemoglobin obtained from patients, one in the high range with a value of 14.6% HbA$_1$c and the other in the low range of 4.5% HbA$_1$c in the following proportions: 4:0, 3:1; 2:2, 1:3, 0:4, as described in protocol EP6-CLSI [14].

Statistical analysis

The methods were compared and the correlation assessed using Passing-Bablok non-parametric regression and calculating Pearson's coefficient of correlation (r) to determine the confidence intervals for $p < 0.05$. The bias was estimated using a Bland-Altman dispersion plot. The coefficients of variation (CV %) to estimate intra- and inter-assay variability were also calculated. The data analysis and statistical calculations were performed using Excel and Method Validator.

RESULTS

The first comparison was between Cobas b101 (Roche Diagnostics, Afinion™ (Alere Technologies), B-analyst (Menarini Diagnostics) and G8 (Tosoh®) as the reference method.

After analyzing the values for glycated hemoglobin in the patient samples, there were no outliers for any of the three units. We are therefore working with 100 samples distributed in four HbA$_1$c ranges: 4% to 6% (20 - 42 mmol/mol), 6% to 8% (42 - 64 mmol/mol), 8% to 10% (64 - 86 mmol/mol) and > 10% (> 86 mmol/mol). Applying Passing-Bablok regression (Table 1, Figure 1) between the Afinion and the G8 results in a slope of 0.967 with a 95% confidence interval (0.938 - 1.000) and a y-intercept: 0.263, confidence interval (0.000, 0.475), and the coefficient of correlation r: 0.984. In the case of B-analyst compared to the G8 results in a slope of 1.036 (1.000, 1.056), y-intercept: -0.14 (-0.30, 0.10), r = 0.996 ($p < 0.05$), and finally in the case of the Cobas b101 the slope was 0.886 (0.865, 0.909), y-intercept: 0.80 (0.61, 0.96), r = 0.99 ($p < 0.05$).
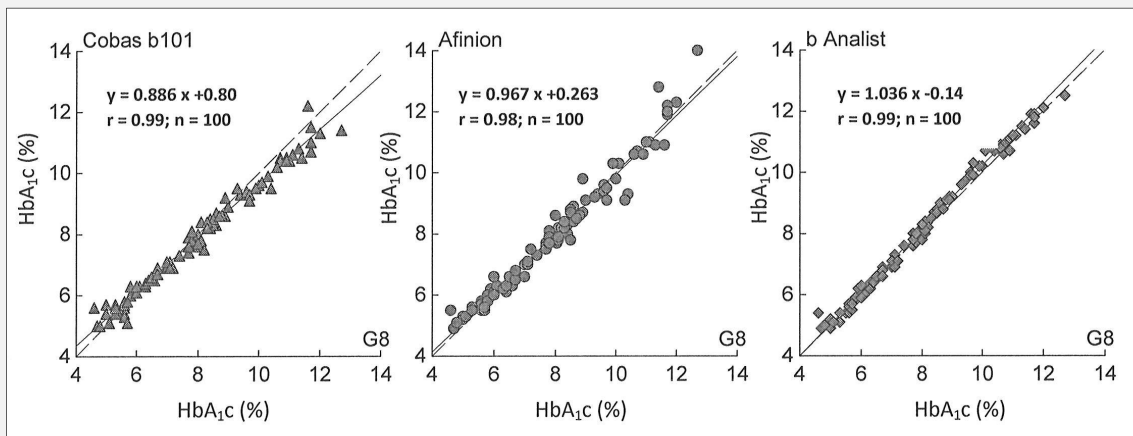
Figure 1. Passing-Bablok plot of the data obtained with Cobas b101, Afinion, and B-analyst compared to G8.
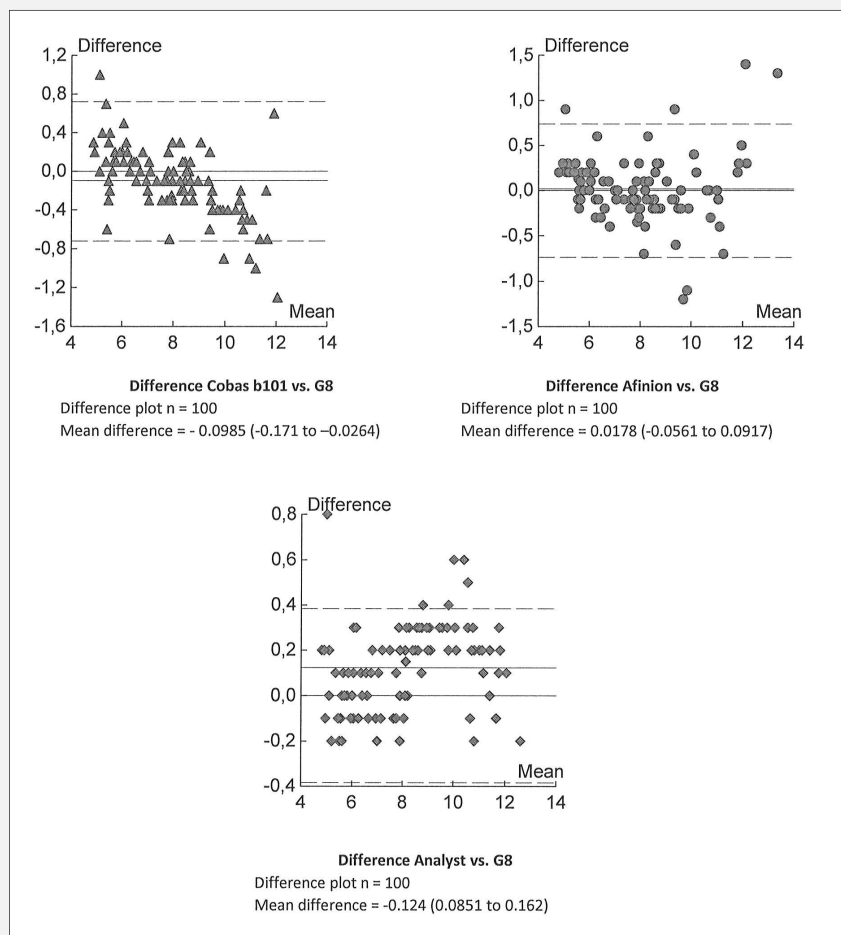

Figure 2. Bland - Altman plot (differences) of the data obtained with Cobas b101, Afinion and B-analyst compared to G8.
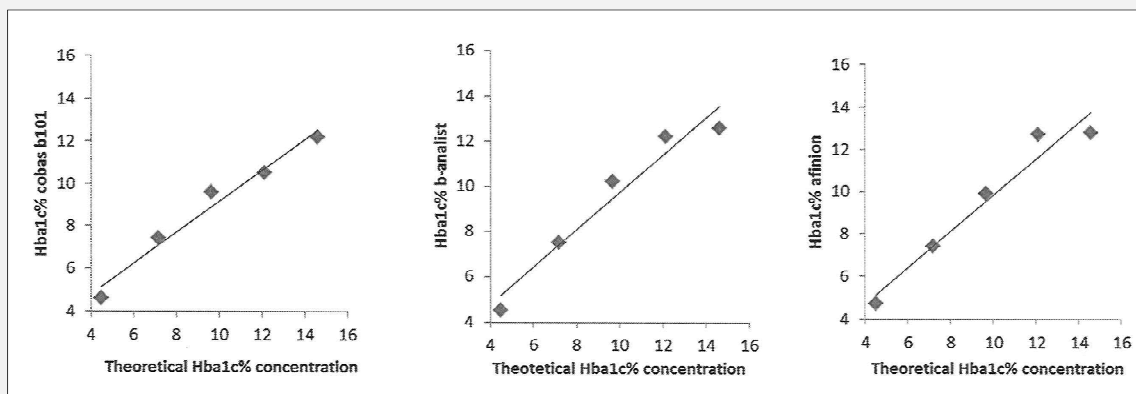
Figure 3. Plot of the linearity of the different methods from data obtained with Cobas b101, Afinion, and B-analyst compared to the theoretical HbA₁c concentrations.

A study of the differences between the HbA₁c measurements is shown in Figure 2 and Table 2. The mean difference between Afinion and G8 was 0.0178 (-0.0561, 0.0917), between B-analyst® and G8 0.124 (0.0851, 0.162), and in the case of the Cobas b101, -0.0985 (-0.0171, -0.0264), in all three cases with a 95% confidence interval.

The values for CV% obtained for Cobas b101, Afinion, and B-Analyst for in intra-assay variability were 1.92, 2.13, and 1.34, respectively, in the case of samples with high HbA₁c value = 12% and in the case of samples with a low HbA1c value = 4.4% they were 2.06, 1.13, and 1.79, respectively. The inter-assay variability for an HbA₁c value of 6.1% were 1.87, 1.97, and 3.17%, respectively, (Table 3).

The three methods studied are linear in the test interval (Figure 3), even though when performing the assessment we noted that for the last dilution, corresponding to the 14.6% HbA₁c high value sample, no value for glycated hemoglobin was obtained with any of the three units.

DISCUSSION

Considerable effort is being made for POC methods for determining HbA₁c toward their technological development and this has resulted in considerable improvements in the analytical performance of these devices, as shown by Lenters-Westra et al. [10].

The results of this study show that the Cobas b101, Afinion, and B-analyst instruments present a good correlation with the reference method. Direct observation of the straight lines plotted using Passing-Bablok regression shows that there is a very high degree of concordance between the three units and good linear correlation with the laboratory reference method. These results agree with those reported earlier for Afinion and B-analyst [10,15,16]. By checking thoroughly the slope and intercept data from the Passing Bablok regression, we can conclude that Afinion is comparable to G8 while b-Analyst and Cobas b 101 are not.

The results obtained after Bland-Altman analysis of the units studied and the reference method shows that the differences are minimal, ranging between -0.0985 in the case of the Afinion and 0.124 in that of the B-analyst, indicating only slight differences between methods. As it can be seen from the Passing Bablok regression as well as the Bland-Altman plot, Afinion is comparable to G8 while b-Analyst and Cobas b101 show a concentration dependent variation.

The units assessed show a within-run imprecision of 2.1% or less for all three analyzers when, according to generally accepted performance criteria for the determination of HbA₁c, an acceptable imprecision is considered to be < 3% [10] with only B-analyst presenting a slightly higher value of 3.1%. Although it is unusual that inter-assay-CVs are lower than intra-assay CVs, the slight differences maybe can be explained by the fact that the samples used to intra-assay CVs had more extreme values (4% and 12 % HbA₁c values) and thus the linearity is weaker than in the case of inter-assay CVs (6.1% HbA₁c value).

Assessment of the performance of the three POC analyzers showed bias and imprecision values compatible with the clinical use of HbA₁c for controlling and monitoring diabetic patients in accordance with NGSP recommendations. Nevertheless, the bias and imprecision are not low enough to recommend the use of POC HbA₁c for diagnostic purposes as suggested by an earlier study by Lenters-Westra et al. [9,10].

CONCLUSION

In summary, the three POC HbA$_1$c devices assessed offer the advantages of fast and reliable test results that making it possible to improve the care of diabetic patients as well as the possibility of establishing early treatment because of their immediate availability. They are therefore considered suitable for the control, but not for the diagnosis of diabetes [15,17].

Ethics:
Due to the study design, there was no need for a statement of ethics.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

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