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Fluorescence Immunoassay versus HPLC: Two Approaches for HbA1c Measurement, Same Reliability?

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Abstract

Objective: To compare the analytical performance of two HbA1c measurement methods: high-performance liquid chromatography (HPLC) using the Turbo Variant II system (Bio-Rad), considered the reference method, and fluorescence immunoassay using the STANDARD F200 system (SD Biosensor).

Methods: Fifty-five EDTA-anticoagulated whole blood samples were analyzed using both techniques. Results were compared using Bland-Altman analysis and Passing-Bablok regression to assess agreement and correlation between the two methods.

Results: The mean HbA1c values were 7.461 ± 2.037% for HPLC and 7.740 ± 2.021% for the STANDARD F200. Bland-Altman analysis showed a mean bias of 0.279%, with limits of agreement ranging from -0.248% to 0.804%. Passing-Bablok regression yielded the equation Y = 0.9837X + 0.3999 with a high correlation coefficient ($r = 0.991$, $p < 0.001$).

Conclusion: Both methods demonstrated good correlation and satisfactory agreement, with a slight overestimation of values by the STANDARD F200. Given its faster processing time and greater accessibility, the STANDARD F200 represents a reliable alternative to HPLC, particularly in settings requiring rapid screening or with limited resources.

Keywords: HPLC; Fluorescence Immunoassay; Method Comparison; HbA1c; Diabetes

1. Introduction

Diabetes mellitus is a major global public health issue, with prevalence continuously increasing [1]. Effective management of this condition largely depends on glycemic monitoring, among which glycated hemoglobin (HbA1c) is a key marker. Recommended by leading scientific societies for the diagnosis and monitoring of diabetes [2], HbA1c measurement reflects the average glycemic control over the past two to three months, independent of daily fluctuations in blood glucose levels [3].

To ensure optimal patient management, it is essential that HbA1c measurement relies on reliable, standardized, and validated methods [4]. High-performance liquid chromatography (HPLC), particularly with the Turbo Variant II (Bio-Rad), is currently considered the reference method due to its precision, specificity for hemoglobin variants, and compliance with NGSP and IFCC standards [5]. However, in many contexts, particularly in resource-limited settings or environments requiring rapid results, alternative methods that are simpler to use and less costly are increasingly being employed. Among these, fluorescence immunoassays, such as the Standard F200 (SD Biosensor) system, offer a

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practical, rapid, and accessible solution. However, their reliability and comparability with the reference method must be rigorously assessed before widespread adoption [6].

The objective of this study is to compare the analytical performance of the Standard F200 system, based on immunofluorescence, with the reference method, HPLC on the Turbo Variant II, for HbA1c measurement. This evaluation will focus on result concordance, precision, and the clinical validity of both approaches.

2. Materials and methods

2.1. Population and Samples

This study was conducted at the medical biology laboratory of the Avicenne Military Hospital in Marrakech. It involved 55 whole blood samples collected from outpatients or hospitalized patients, both diabetic and non-diabetic, as part of routine HbA1c testing. The samples were collected in vacutainer tubes containing EDTA (5 mL), in accordance with standardized preanalytical recommendations [7]. No specific exclusion criteria were applied, and the analyses were performed on fresh samples within a time frame compatible with the stability of HbA1c [8].

2.2. Analytical Methods

2.2.1. HbA1c Measurement by HPLC (Reference Method)

HbA1c measurement by high-performance liquid chromatography (HPLC) was performed using the Turbo Variant II analyzer (Bio-Rad). This automated system uses an ion-exchange technique to separate different hemoglobin fractions on a thermostable column [9]. An increasing ionic strength gradient is applied to separate the molecules based on their ionic interaction, with detection of peaks at 415 nm using a light-emitting diode photometer.

The results are processed using the Clinical Data Management (CDM™) software, which calculates the HbA1c percentage from a two-level calibration curve [10]. Each analysis is accompanied by a chromatogram to assess the quality of separation and identify potential variants.

2.3. HbA1c Measurement by Fluorescence Immunoassay (Tested Method)

The Standard F200 system is an automated in vitro diagnostic device based on immunoassay technology coupled with reflectometry, allowing for the quantification of HbA1c from a sample of capillary or venous whole blood [11].

The principle relies on the specific recognition of the glycated form of hemoglobin A0 (HbA1c) by a monoclonal antibody targeting the first glycosylated N-terminal residues of the β-chain of hemoglobin. During the test, the blood sample is mixed with an extraction buffer containing lysis agents that induce rapid release of intracellular hemoglobin.

Meanwhile, a latex tablet releases colored microparticles conjugated to specific anti-HbA1c antibodies. The mixture reacts, resulting in an immunological complex proportional to the amount of HbA1c in the sample. This complex is then capillary-migrated along a test membrane to a zone where immobilized capture antibodies are located.

2.3.1. Two readings are taken

- The intensity of the blue color formed on the HbA1c detection line, proportional to the bound HbA1c concentration;
- The total hemoglobin quantity, measured in a distinct area of the device via reflected optical signals.

The Standard F200 analyzer captures these signals using an optical system for fluorescence and reflectometry detection. An embedded algorithm then calculates the HbA1c percentage by dividing the HbA1c quantity by the total hemoglobin, in accordance with international standards (IFCC/NGSP).

This method provides a rapid, automated, and standardized result, particularly suitable for low-throughput settings or rapid screening contexts, although precision may be affected by certain hemoglobin variants or analytical interferences [12].

2.4. Quality Control

An internal quality control program was implemented throughout the study. Control materials used were those provided by the respective manufacturers of the two systems (Bio-Rad and SD Biosensor). The instruments were handled and calibrated according to the instructions in the user manuals provided.

2.5. Statistical Analysis

The data obtained were processed using Microsoft Excel software (version 2019). The correlation between the two methods was studied using Passing-Bablok regression, while concordance was assessed using the Bland-Altman method, which allows for analysis of individual differences between the two systems. The statistical significance threshold was set at $p \leq 0.05$.

This study was conducted in accordance with the ethical standards of the institution. Patient confidentiality was respected and all samples were anonymized prior to analysis.

3. Results

The mean HbA1c values obtained by the two methods were 7.461 ± 2.037% for the HPLC method (Bio-Rad Turbo Variant II) and 7.740 ± 2.021% for the fluorescence immunoassay method (Standard F200). These data indicate a slight average difference between the two techniques, but within close ranges.

To evaluate the concordance between the two methods, a Bland-Altman graphical analysis was performed [13]. This method allows for the comparison of differences between the results obtained by the two techniques relative to their means. According to the Bland-Altman plot, the differences between the HPLC and F200 measurements were within a ±1.96 standard deviation (SD) interval, indicating that the two methods can be used interchangeably in most cases. However, two outliers were identified, which fell outside this interval, leading to their exclusion from the final analysis. The mean bias observed between the two methods was 0.278%, suggesting very good concordance between the values measured by the two systems. The plot also shows that the difference between HbA1c values measured by HPLC and F200 was within a range of -0.248% to 0.804%.

The Passing-Bablok regression [14] showed a very strong correlation between the results of the two techniques, with a correlation coefficient (r) of 0.991. This indicates an almost perfect linear relationship between the values obtained by HPLC and those obtained by the Standard F200 system. The regression equation is as follows: Y (F200) = 0.3999 + 0.9837X (Bio-Rad Variant II), meaning that results obtained by F200 can be predicted with high accuracy from the results obtained by the HPLC method. The coefficient of determination $r^2 = 0.9826$ shows that 98.26% of the variance in F200 results can be explained by the HPLC method. This high r^2 value strengthens the idea that both methods essentially measure the same thing. The p-value < 0.005 indicates that the observed relationship is statistically significant, confirming the reliability of the regression and the robustness of the comparison between the two methods.

Table 1 Criteria for Comparing the Results of the Two Methods

Method	Mean	SD	Minimum	Maximum
Standard F200	7,740	2,021	5,300	13,300
Bio-Rad Turbo VARIANT II	7,461	2,037	4,800	13,100

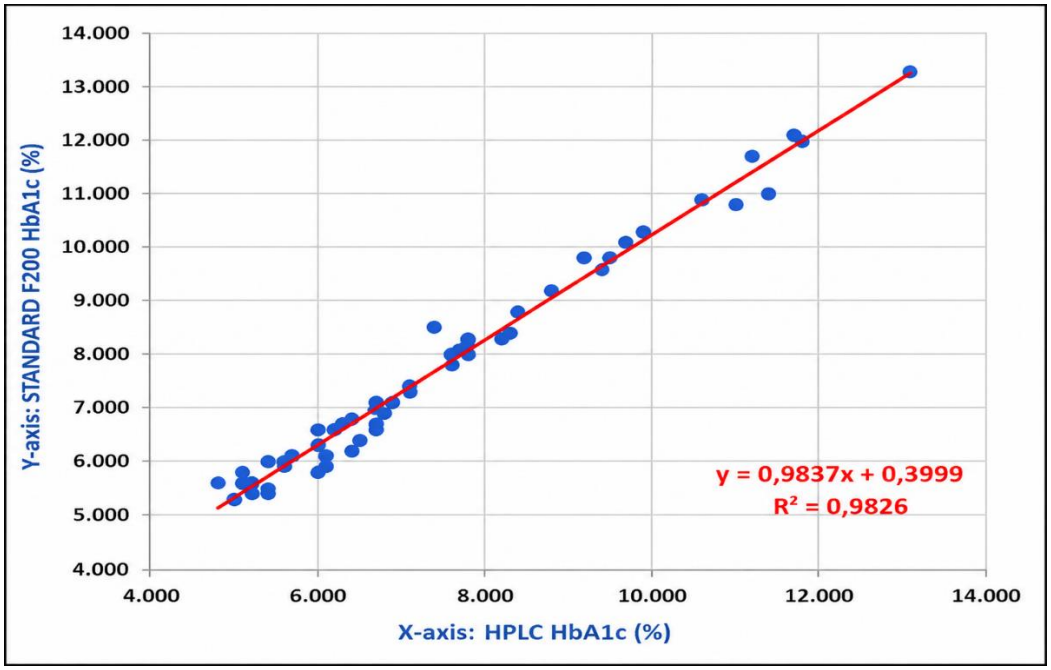

Figure 1 Passing-Bablok regression for both HbA1c assay techniques

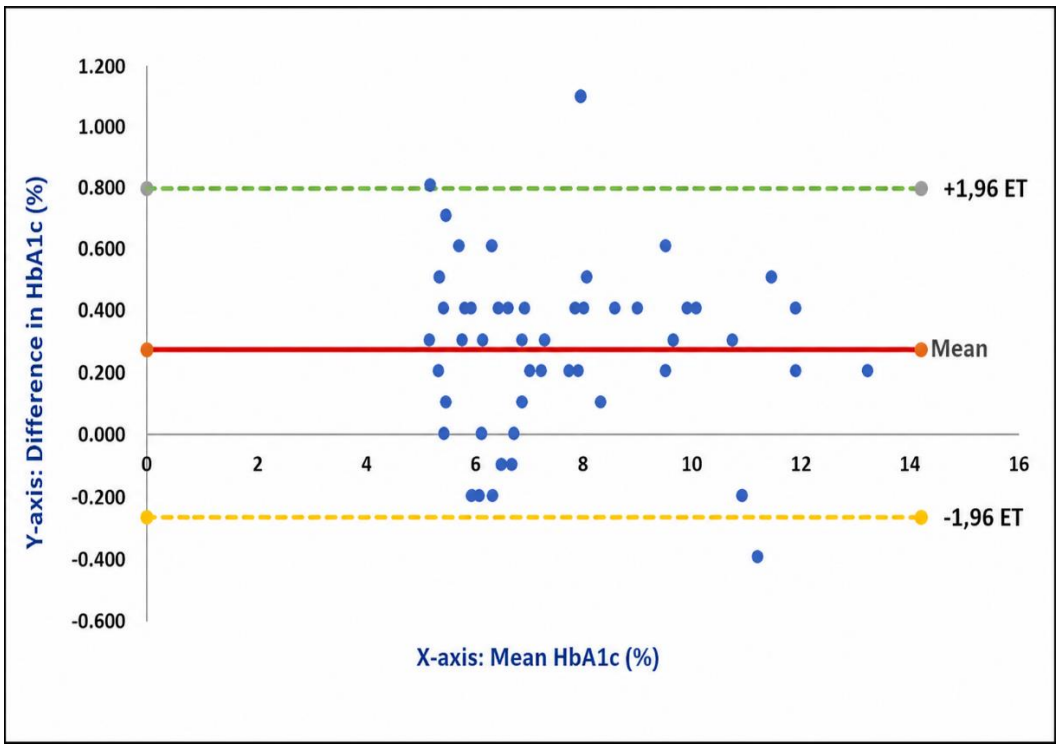

Figure 2 Bland-Altman plot comparing both HbA1c assay techniques

4. Discussion

The use of HbA1c for the screening and diagnosis of diabetes has gained increasing importance in recent years, following validation by numerous studies, such as DCCT and UKPDS [15]. These studies established that HbA1c is a reliable and robust indicator for long-term glycemic monitoring, and it has been recommended by institutions such as the American Diabetes Association to complement the clinical profile of the patient in the context of diabetes diagnosis [16]. However,

to ensure the validity of clinical results, it is imperative that HbA1c measurement be performed using certified and rigorously controlled methods, such as those employed in reference clinical studies. In this study, the HPLC method (Bio-Rad Turbo Variant II) was used as the reference method, compared to the fluorescence immunoassay method STANDARD F200.

The results obtained showed that, although both methods provide similar values, there is a slight difference between the mean HbA1c values measured by each of them. Specifically, the HPLC method gave a mean of 7.461 ± 2.037 %, while the STANDARD F200 method showed a mean of 7.740 ± 2.021 %. The average difference between the two methods was 0.279 ± 0.016 % ($p < 0.005$), indicating that the STANDARD F200 method tends to slightly overestimate HbA1c values compared to the HPLC method. Although this difference is statistically significant, it remains small and within clinically acceptable limits.

It is important to note that, in the follow-up of diabetic patients, an absolute difference of 0.5 % (5 mmol/mol) between two HbA1c measurement results is generally considered a clinically significant change in glycemic control [17]. In our study, the 95 % confidence interval (CI) for the differences between the two methods ranged from -0.24 % to 0.80 %, with a total range of 0.56 %, which could indeed have clinical implications in certain glycemic monitoring situations. However, given the high correlation between the two methods ($r = 0.991$), this suggests that the variation between the results is not large enough to cause a significant clinical error in therapeutic decisions, provided that this slight difference in measured values is taken into account.

The results of our study showed a very strong correlation between the two methods, as indicated by the regression equation $y = 0.9837x + 0.3999$ ($p < 0.001$). This high correlation suggests that, although both methods have very similar analytical performances, the STANDARD F200 method can be used as a reliable alternative to the HPLC method for measuring HbA1c. These results are consistent with those of Michel Vogeser et al., who also observed perfect agreement between an automated immunological test and the HPLC method in their study in Germany, with a correlation coefficient of $r = 0.99$ and identical mean results for both techniques [18].

Our results also indicate that both methods have equivalent analytical performance, with inter-test coefficients of variation of around 1 %. This complies with the quality standard recommended for HbA1c assays, which stipulates that the long-term coefficient of variation must be less than 2 % to ensure the reliability of results. This finding underscores that, in terms of analytical performance, the STANDARD F200 system is fully competitive with the HPLC method, and both can be considered reliable for HbA1c assays in a clinical setting.

However, despite the good agreement between the two methods, further studies are necessary to evaluate the long-term impact of using the F200 in the clinical management of diabetes, particularly regarding the management of diabetes-related complications. It would also be pertinent to conduct research on the impact of external factors (e.g., environmental or biological variations) on the performance of HbA1c assay methods.

5. Conclusion

This study demonstrated that the HbA1c measurement methods by HPLC (Bio-Rad Turbo Variant II) and fluorescence immunoassay (STANDARD F200) are highly correlated and produce comparable results. The STANDARD F200, due to its speed, simplicity, and reduced cost, presents a reliable and accessible alternative to the HPLC method, particularly for high-volume laboratories or those with limited resources. These results support the idea that HbA1c, measured by both techniques, is an effective tool for screening and managing diabetes, while promoting optimal glycemic monitoring.

In conclusion, the integration of faster and more accessible methods like the STANDARD F200 could improve access to diagnosis and monitoring of diabetic patients, thereby contributing to more effective diabetes management.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

This study was conducted in accordance with institutional ethical standards. Patient confidentiality was respected and all samples were anonymized prior to analysis.

Statement of informed consent

As the study was performed on anonymized biological samples collected during routine laboratory practice, informed consent was not required.

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