Research Article

Comparison of two analytical methods for HbA1c determination: HPLC ADAMS™ (ARKRAY A1c HA-8180T) versus CAPILLARYS 3 OCTA® (Sebia) capillary electrophoresis system

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Abstract

HbA1c is a valuable indicator for the diagnosis and therapeutic monitoring of diabetic patients. Our study aims to compare two methods of measuring HbA1c: capillary electrophoresis on the CAPILLARYS 3 OCTA ® (Sebia) with HPLC ADAMS™ (ARKRAY A1c HA-8180T) used routinely in our laboratory, to avoid any discrepancies in patient monitoring in case of changes in the HbA1c measurement method.

A total of 103 blood samples from adult patients received at the laboratory have been analyzed in parallel, singly, on both machines. The results show a good correlation between the two systems with a correlation coefficient of 0.991. The Bland and Altman difference diagram shows that the average bias between ADAMS A1c™ and CAPILLARYS 3 OCTA ® is 2.087 mmol/mol (95% CI: 1.7357 to 2.4390 mmol/mol) in IFCC units and 0.19% (95% CI: 0.1602 to 0.2262%) in the National Glycohemoglobin Standardization Program (NGSP) units, and that out of all the patients studied, only four had values outside the limits of the difference diagram. The latter shows a uniform dispersion of values across all the analyzed measurements, with over 95% of the differences between measurements falling within the range [-1.43; 5.61 mmol/mol] or [-0.14; 0.52%].

These results enable us to confirm the reliable transferability between the two techniques without compromising accuracy. Both machines can therefore be used interchangeably or as backup, ensuring homogeneous patient monitoring.

Introduction

Glycated hemoglobin corresponds to the main glycosylated portion of hemoglobin. This process results from the non-enzymatic and irreversible binding of glucose to one or two of the N-terminal valines of the β chain of hemoglobin. In the absence of interfering factors, the measurement of HbA1c reflects an individual's glycemic control over the 2 to 3 months preceding the sample collection [1] .

This places this parameter at the core of both the diagnosis and monitoring of diabetic patients, as well as the assessment of the risk of complications, as demonstrated by various large-scale studies such as the Diabetes Control and Complications Trial (DCCT) for type 1 diabetics and the United Kingdom Prospective Diabetes Study (UKPDS) for type 2 diabetics. These studies have established a correlation between controlled HbA1c levels and a reduced risk of long-term complications, particularly microvascular complications [2–4].

Several analytical methods are currently available for the measurement of HbA1c (chromatographic, electrophoretic, and immunochemical methods). The National Glycohemoglobin Standardization Program (NGSP) recognizes high-performance liquid chromatography (HPLC) using cation exchange as the reference method. Meanwhile, a working group of the International Federation of Clinical Chemistry (IFCC) has established a reference procedure based on reverse-phase HPLC coupled with mass spectrometry or capillary electrophoresis [5,6].

In the present study, we compared two analytical methods for the measurement of HbA1c :high-performance liquid chromatography (HPLC) using ion exchange and capillary electrophoresis.

The objective is to assess the transferability of results between the two automated systems, thereby ensuring consistency in patient monitoring when a change in the HbA1c measurement method occurs. This would allow both systems to be used in parallel or as backup instruments within the laboratory. Given the limited number of published evaluations of the recently introduced CAPILLARYS 3 OCTA system, this work provides timely and relevant data for clinical laboratories seeking to harmonize HbA1c measurements.

Materials and Methods

Patient Samples

The study included 103 venous whole-blood samples collected from adult patients referred to the biochemistry laboratory for HbA1c testing as part of diabetes diagnosis or follow-up. Given the pre-analytical stability of HbA1c, fasting was not required. Venous whole blood samples were collected in 4 ml EDTA Vacutainer tubes (Becton Dickinson) from both the hospital wards and the laboratory's reception and sampling center. The collected samples were stored at room temperature and analyzed in parallel on both automated analyzers on the same day of collection. The 103 patients included in our sample covered the measurement range defined by the manufacturer and exhibited normal hemoglobin chromatographic profiles.

Devices and measurement techniques

HbA1c measurements were compared using two automated systems: the HPLC-based ADAMS™ (ARKRAY A1c HA-8180T) and the recently implemented CAPILLARYS 3 OCTA® (Sebia).

The HPLC ADAMS™ (ARKRAY A1c HA-8180T) uses a high-performance liquid chromatography (HPLC) technique based on ion exchange, which relies on inorganic phosphate buffers (80 A, 80 B, and 80 CT) with increasing ionic strengths, which elute the different glycated and non-glycated fractions of the hemoglobin being analyzed. These fractions are then quantified by spectrophotometry using a dual wavelength (420/500 nm) [7] . This instrument provides a reportable HbA1c measurement range of 9–195 mmol/mol using IFCC units, corresponding to 3–20% on the NGSP scale, while the guaranteed analytical performance range extends from 22 to 117 mmol/mol , equivalent to 4.2 to 12.9 % [8] .

The CAPILLARYS 3 OCTA® system (Sebia), on the other hand, employs an automated capillary electrophoresis technique. This method is based on the separation of different hemoglobin fractions inside capillaries with very small diameters, subjected to a potential difference of several thousand volts at their ends. The fractions are detected directly on the cathode side at a wavelength of 415 nm [9] . Thanks to its high resolution, this method allows for the incidental detection of homozygous hemoglobin variants, which prevents erroneous HbA1c reporting in the absence of HbA. Analytical performance studies have reported that this analyzer provides linear HbA1c measurements across an approximate range of 25–199 mmol/mol , corresponding to 4.4–20.3% [10].

The results delivered by the two automated systems are expressed in NGSP (%) and IFCC (mmol/mol) units linked by the following equation:

HbA1c (NGSP/DCCT in %) = HbA1c (IFCC in mmol/mol) x 0.0915 + 2.152

Protocol and Statistics

A prospective analytical study was conducted on patient samples referred to the biochemistry laboratory for HbA1c testing, in accordance with the ethical principles outlined in the Declaration of Helsinki. Each sample was analyzed in series, with a single measurement per method, on the day of receipt. Data entry was performed using Excel 2016, and statistical analysis of the data was performed using MedCalc® software and jamovi (version 2.7.17). The study design followed the recommendations of the SH GTA 04 – Revision 02 protocol of the French Accreditation Committee (COFRAC) [11] .

The bias, concordance, and correlation between the two methods were assessed using the Bland-Altman plot and the Passing-Bablok and Deming regression tests.

Additional agreement analyses were conducted at clinically relevant HbA1c cutoffs of 39 mmol/mol (5.7%), 48 mmol/mol (6.5%), and 53 mmol/mol (7.0%). For each cutoff, samples

within ±3 mmol/mol (±0.3%) of the target value were selected to focus on the clinically critical range. Local bias between the two methods was determined, along with the corresponding standard deviation and 95% confidence interval. Classification agreement was assessed by categorizing results according to each cutoff and calculating the proportion of concordant classifications, with Cohen's kappa coefficient and McNemar's test.

Results

The study included 103 adult patients (60 females and 43 males; male-to-female ratio: 0.72). The mean age of the participants was 56.9 ± 16.1 years. The mean HbA1c values delivered by the two methods were 51.24 ± 18.26 mmol/mol (or 6.84 ± 1.66%) for the ADAMS A1c™ and 49.15 ± 17.88 mmol/mol (or 6.64 ± 1.63%) for the CAPILLARYS 3 OCTA®. Although CAPILLARYS 3 OCTA® produced slightly lower mean values, the difference was not clinically significant.

The equation of the Passing-Bablok regression (Figure 1) yielded the following equation:
Y (CAPILLARYS 3 OCTA ®) = -2.000000 + 1.000000 X (ADAMS A1c)
Similarly, the Deming regression produced the equation:
Y (CAPILLARYS 3 OCTA ®) = -1.0075 + 0.9789 X (ADAMS A1c)
The correlation coefficient was 0.991 ($P < 0.0001$).

Figure 1: Comparison between the two methods using the Passing and Bablok regression line.

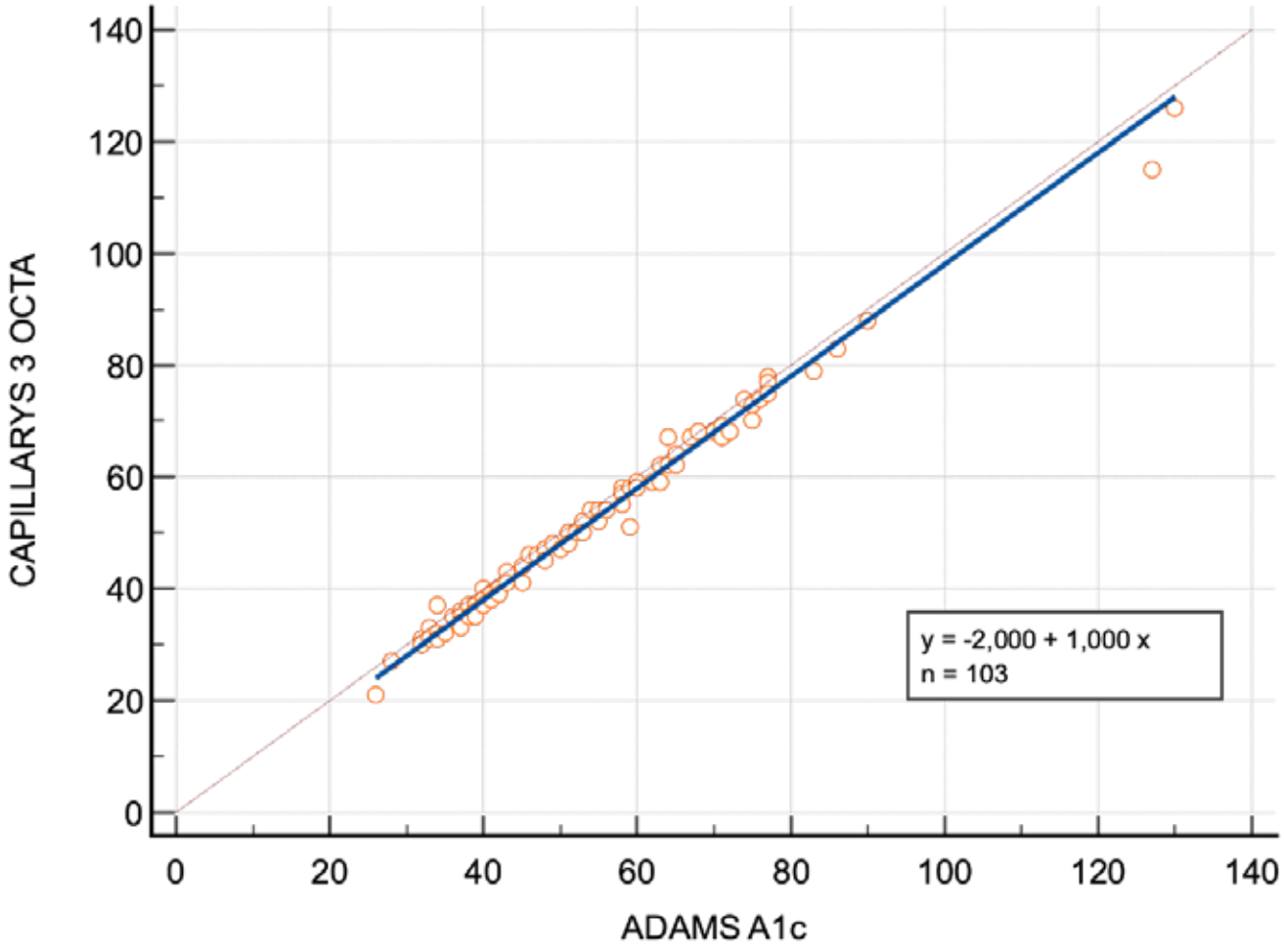
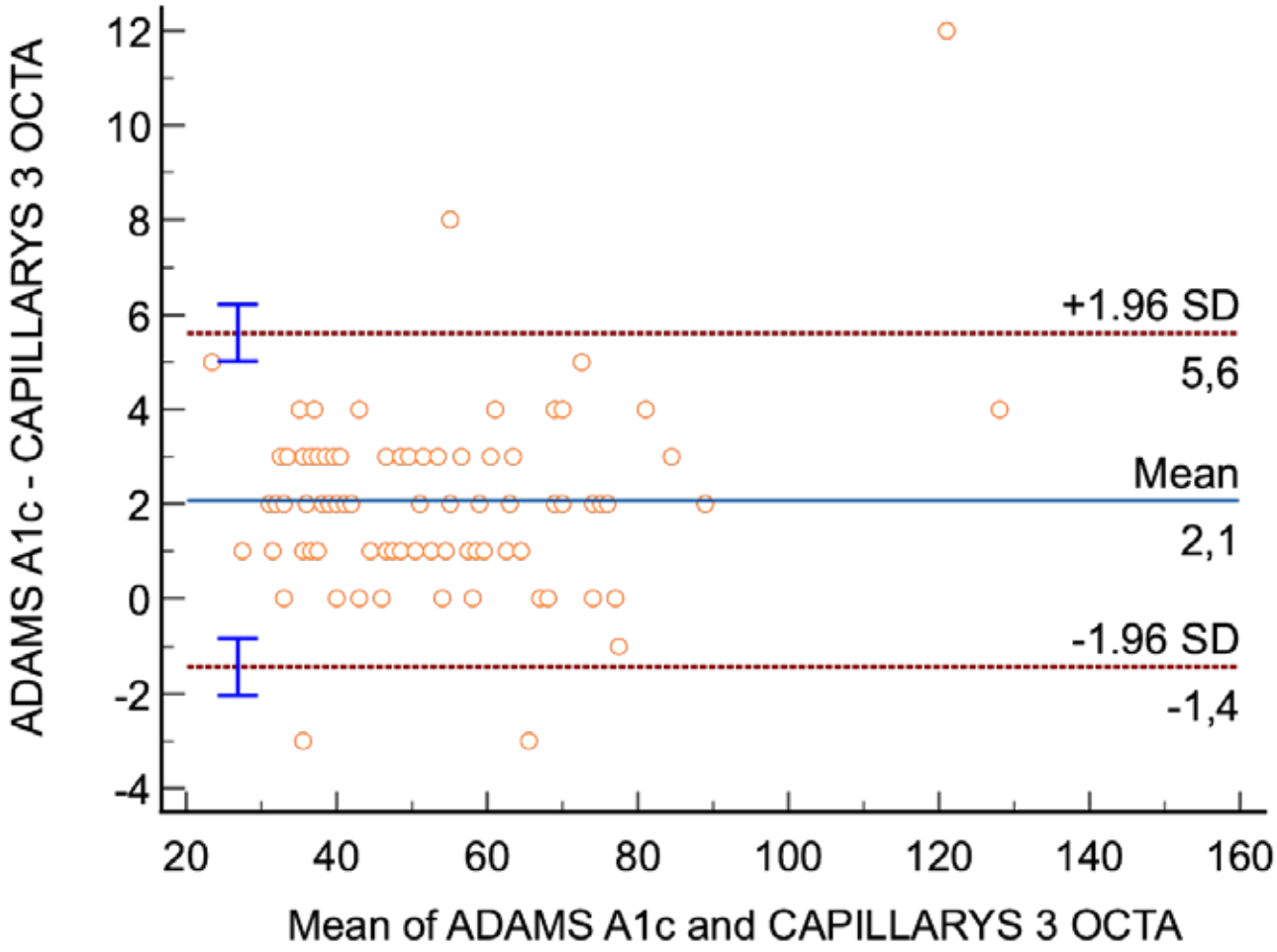


Figure 2: Comparison between the two methods using the Bland-Altman plot.



The Bland - Altman analysis revealed a mean bias of 2.09 mmol/mol (95 % CI: 1.74–2.44 mmol/mol) in IFCC units and 0.19 % (95 % CI: 0.1602–0.2262 %) in NGSP units between the ADAMS™ A1c HA-8180T and CAPILLARYS 3 OCTA® systems.

Among the 103 patients included in the study, only 3.8 % (4 patients) exhibited values falling outside the limits of the difference plot. The difference between HbA1c measurements using the two methods ranged from +1.96 SD to -1.96 SD, meaning that 95% of the differences between measurements fell within the range [-1.43; 5.61 mmol/mol] or [-0.14; 0.52%], respectively, in IFCC and NGSP units.

The agreement between the two methods was assessed at clinically relevant HbA1c cutoffs of 39 mmol/mol (5.7%), 48 mmol/mol (6.5%), and 53 mmol/mol (7.0%) (±3 mmol/mol). The mean biases were 2.21 mmol/mol (SD 0.91, 95% CI 1.89–2.52, N = 34), 1.83 mmol/mol (SD 1.27, 95% CI 1.03–2.64, N = 12), and 2.00 mmol/mol (SD 1.04, 95% CI 1.40–2.60, N = 14), respectively.

Classification concordance was high across all cutoffs, with 91.3% agreement at 39 mmol/mol (5.7%) (Cohen's $\kappa = 0.81$; McNemar $\chi^2 = 9.00$, $p = 0.003$), 96.1% at 48 mmol/mol (6.5%) ($\kappa = 0.92$; McNemar $p > 0.05$), and 95.1% at 53 mmol/mol (7.0%) ($\kappa = 0.90$; McNemar $\chi^2 = 5.00$, $p = 0.025$).

Discussion

HbA1c measurement is essential in the management of diabetic patients. However, the variety of analysis methods available on the market can lead to variations in the HbA1c values obtained. That is why it is essential to continue efforts to standardize and improve quality in order to guarantee reliable results and reduce inter-technical disparities.

Comparing methods is an essential part of the analytical evaluation of a measurement method. It allows the results produced by these methods to be compared and any potential bias between them to be identified. In the event of divergent results, it is crucial to investigate the causes and find appropriate solutions [5,12].

In our study, the mean HbA1c values for all samples measured by capillary electrophoresis were 49.15 ± 17.88 mmol/mol (6.64 ± 1.63%), slightly lower than those obtained by high-performance liquid chromatography (HPLC), which were 51.24 ± 18.26 mmol/mol (6.84 ± 1.66%). However, the difference between the average HbA1c results obtained by

HPLC on the ADAMS A1c™ and CAPILLARYS 3 OCTA® was only 0.2%, remaining well below the 1.4% acceptability threshold established by the French Health Products Safety Agency (AFSSAPS) guidelines. This result demonstrates the effectiveness of efforts to standardize HbA1c testing, even when the analytical methods rely on distinct measurement principles.

The Bland-Altman plot analysis of agreement between ADAMS A1c™ and CAPILLARYS 3 OCTA® was satisfactory. The mean bias was 2.087 mmol/mol (equivalent to 0.19%) between the two methods. The most significant differences were observed for normal and low HbA1c values as well as for very high values (>119 mmol/mol).

The difference diagram shows a uniform dispersion of values across all measurements analyzed, with more than 95% of differences falling within the limits of agreement. These limits range from [-1.43 to 5.61 mmol/mol] or [-0.14% to 0.52%] in IFCC and NGSP units, respectively.

Of the 103 samples analyzed, four showed a weak correlation between the results obtained on the two devices. These samples had HbA1c values ranging from 21 mmol/mol (4.1%) on the CAPILLARYS 3 OCTA® to 26 mmol/mol (4.6%) on the ADAMS A1c™ HPLC system, and between 115 mmol/mol (12.7%) on the CAPILLARYS 3 OCTA® and 127 mmol/mol (13.8%) on the ADAMS A1c™ automated system.

The discrepancies observed between the two methods, although limited in number, highlight the existence of occasional differences in HbA1c measurement between high-performance liquid chromatography (HPLC) and capillary electrophoresis. These differences may result from various methodological, biological, pre-analytical, or instrumental factors.

However, in our series, no major analytical interference was identified. Fetal hemoglobin (HbF) levels were within normal limits, no hemoglobin variants were detected, and no history of anemia or renal failure was reported in the patients concerned. These factors rule out the usual biological causes that could explain such a disparity between the two techniques.

Thus, the differences observed could be attributed to variations intrinsic to the analytical methods themselves, in particular differences in calibration, detection sensitivity, or signal processing between capillary electrophoresis systems.

Regarding correlation analysis, the two methods compared showed a strong correlation, with a correlation coefficient of 0.991. The Passing-Bablok equation establishes that: Y (CAPILLARYS 3 OCTA®) = -2.000000 + 1.000000 X (ADAMS A1c). Looking at the 95% confidence interval for the slope coefficient, we see that it encompasses the value of one. This indicates that there is no statistically significant difference between the slope value obtained and the value of one. Therefore, we can conclude that there is no significant proportional difference between the two methods [13].

In method comparison studies, global analytical agreement may mask clinically significant differences when measurements fall near decision thresholds that directly influence diagnosis and treatment. Therefore, particular attention was given to clinically relevant HbA1c cutoffs of 39 mmol/mol (5.7%), 48 mmol/mol (6.5%), and 53 mmol/mol (7.0%), which correspond to prediabetes identification, diabetes diagnosis, and commonly recommended therapeutic targets, respectively [14,15] . Assessing agreement specifically at these decision levels provides a more clinically meaningful evaluation of interchangeability between HbA1c measurement systems than overall performance metrics alone.

Even minor systematic bias or increased variability around these thresholds,particularly the diagnostic cutoff of 48 mmol/mol,may lead to patient misclassification and inappropriate clinical decisions. Similarly, discrepancies near the prediabetes or therapeutic targets may influence risk stratification and treatment adjustment.

Assessment of agreement at the clinically relevant HbA1c decision thresholds of 39, 48, and 53 mmol/mol demonstrated a small, consistent positive mean difference of approximately 2 mmol/mol. The stability of this deviation across all evaluated levels, together with its narrow confidence intervals, suggests a systematic yet limited analytical difference rather than random fluctuation. Notably, this magnitude remains well within the clinically acceptable limit of 5 mmol/mol (approximately 0.46% NGSP), in accordance with IFCC defined analytical performance specifications [16] .

Classification agreement was high at all three decision points, with concordance exceeding 90% and Cohen's kappa coefficients indicating strong to almost perfect agreement. Although the McNemar test reached statistical significance at 39 and 53 mmol/mol, this likely reflects a small but systematic directional shift rather than substantial clinical misclassification. At the 48 mmol/mol cutoff, which corresponds to the diagnostic threshold for diabetes, the absence of significant discordance further supports the clinical interchangeability of the two methods in routine practice.

Numerous previous comparisons of automated systems using similar techniques have been conducted. Sriwimol et al. [13] compared HbA1c values obtained by capillary electrophoresis on the Capillarys 3 Tera system (Sebia) with HPLC on the ADAM TM A1c HA-8180V (Arkray) on 270 samples from subjects with normal hemoglobin profiles and no detectable hemoglobin variants on the electropherograms or chromatograms. The results of this study showed good agreement, highlighting a significant consistency and correlation between the two methods, indicating that they are comparable and interchangeable.

The work carried out by Khashoggi et al. [17] also compared high-performance liquid chromatography (HPLC) on Tosoh HLC-723 G11 in VAR mode with a capillary electrophoresis instrument (Sebia Capillarys 2 Flex Piercing). The analysis covered 250 randomly selected patient samples, including some hemoglobin variants (HbS, HbE, and HbD). The results revealed a significant correlation (r = 0.99). However, the Tosoh HLC-723 G11 yielded higher values in the low HbA1c

range and lower values in the high range, making it difficult to use these two automated systems interchangeably in the monitoring of diabetic patients.

Nevertheless, Dupuy et al. [18] remains one of the few studies to our knowledge that has compared the CAPILLARYS 3 OCTA® system recently launched by Sebia with an HPLC system: the Tosoh Bioscience HLC®-723G8. They successfully demonstrated a good correlation between the two methods (r= 0.995), which is consistent with the results obtained in our comparison.

A limitation of our work lies in the absence of hemoglobin variants among the analyzed samples, which prevented assessment of potential interferences caused by abnormal hemoglobins. This aspect warrants further investigation in future studies to evaluate the performance of both systems in the presence of hemoglobinopathies.

Conclusion

Our study demonstrates good agreement between HbA1c results delivered by ADAMS™ (ARKRAY A1c HA-8180T) and CAPILLARYS 3 OCTA®. These results allow us to confirm reliable transferability between the two techniques without loss of accuracy. The consistency observed at major diagnostic and therapeutic decision thresholds further supports their comparability in routine clinical practice. The two automated systems can be used in parallel or as backups while maintaining consistent patient monitoring. This study is part of a quality policy required by the ISO EN 15189 standard, to which medical laboratories must be fully committed.

Conflict of interest disclosure

The authors declare no conflicts of interest.

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Credit Author Statements

Nisma Douzi: Conceptualization, Methodology, Writing - Original Draft, Visualization.Oussama Grari : Writing - Review & Editing, Visualization . Imad-Eddine El Khamlichi: Formal analysis. Soufiane Beyyoudh: Formal analysis. Dounia El Moujtahide: Supervision. El-houcine Sebbar: Resources, Supervision. Mohammed Choukri : Resources, Supervision.

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