

PRECISION and Concordance Analysis of Capillary Electrophoresis and Two Different Methods for HbA1c Measurement among Diabetic and Non- Diabetic Population in Cagayan De Oro CITY

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ABSTRACT

This study aimed to evaluate the precision and concordance of Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence Immunoassay for Hemoglobin A1c (HbA1c) measurement among diabetic and non-diabetic individuals in Cagayan de Oro city. Employing a quantitative, descriptive and comparative research design, 30 archived blood samples were categorized into three glycemic levels: <6.5%, 6.5–8.0%, and >8.0%, using stratified random sampling. The instruments included Sebia Capillarys 2 flex piercing (Capillary Electrophoresis), Bio-Rad D-10 hemoglobin analyzer (High-Performance Liquid Chromatography), and Finecare™ HbA1c rapid quantitative test (Fluorescence Immunoassay). Statistical tools such as Passing-Bablok regression, Bland-Altman plots, and Spearman correlation coefficients were used to assess precision and concordance. Findings showed that Capillary Electrophoresis and High-Performance Liquid Chromatography exhibited high precision and strong concordance, making them reliable for routine clinical use. Fluorescence Immunoassay displayed significant variability at extreme HbA1c levels, limiting its applicability. The study concludes that Capillary Electrophoresis and High-Performance Liquid Chromatography are optimal for HbA1c monitoring, ensuring reliable glycemic control.

KEYWORDS: *Medical Technology, capillary electrohoresis, high-performance liquid chromatography, fluorescence immunoassay, Philippines*

INTRODUCTION

Ensuring the precision and concordance of Hemoglobin A1c (HbA1c) measurements is critical for achieving accurate and reliable laboratory results

and optimization of long-term glycemic management. Despite baseline standardization guidelines framework established by the International Federation of Clinical Chemistry (IFCC) and the National Glycohemoglobin Standardization Program (NGSP), challenges persist in achieving universal cross-platform uniformity. Variability in clinical results has been observed due to fundamental differences in analytical principles and performance, which can compromise the reliability and reproducibility of HbA1c testing across laboratories (English, 2017).

Globally, studies have demonstrated discrepancies in HbA1c measurements across various methodologies. Research by Sriwimol et al. (2019) highlighted that Capillary Electrophoresis (CE) and High-Performance Liquid Chromatography (HPLC) exhibit strong concordance with IFCC standards, whereas Fluorescence Immunoassay (FIA) demonstrates higher variability, particularly at extreme HbA1c levels. Similarly, Gilani et al. (2020) emphasized the need for consistent calibration of HbA1c methods to address inter-laboratory variations. To address these variations, understanding individual platform performance is vital; High-Performance Liquid Chromatography (HPLC) is widely recognized for its high precision and reproducibility, while Capillary Electrophoresis (CE) has gained attention for its ability to produce reliable results that closely align with international reference standards (Liddy et al., 2022). Meanwhile, Fluorescence Immunoassay (FIA) offers a more accessible and cost-effective approach, but concerns about its variability have been raised in multiple studies, especially in the context of inter-laboratory comparisons (Suliman, 2020).

However, a critical research gap persists within the Philippine healthcare landscape, where localized laboratory practices vary widely. In the Philippines, while IFCC and NGSP standardization guidelines are emphasized, achieving precision and concordance across laboratories remains a challenge (Unite for Philippine Diabetes, 2014). Crucially, the Philippine Practice Guidelines (2014) have acknowledged a lack of comprehensive studies comparing HbA1c measurement methods, leaving laboratories without sufficient evidence to guide the selection of appropriate techniques. This problematic situation is highly pronounced in local settings such as Cagayan de Oro City. Despite the use of globally recognized methods like HPLC, CE, and FIA, local evidence evaluating their comparative performance in terms of precision and concordance is insufficient (Philippine Practice Guidelines, 2014). Consequently, local clinical facilities operate without empirical, data-driven regional benchmarks, leaving

them vulnerable to inter-laboratory result divergence that could impact cross-facility patient tracking.

To address this specific diagnostic vulnerability, this study evaluates and compares the performance of Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence Immunoassay, with the goal of establishing definitive benchmarks tailored to the needs of laboratories in Cagayan de Oro City. By evaluating these methods, this research seeks to identify the most reliable and consistent approach for HbA1c measurement, enabling laboratories to adopt best practices in standardization and method selection. This is particularly significant for ensuring reliable and reproducible results across different laboratories.

The findings will be disseminated to medical laboratory scientists and doctors in clinical laboratories and healthcare institutions in Cagayan de Oro City to promote improved practices and standardization of HbA1c testing methods. Furthermore, the results will be shared at research conferences and will be published in academic journals, facilitating the professional presentation of HbA1c measurement standardization both locally and internationally for wider dissemination.

Statement of the Problem

1. What is the mean level of HbA1c measurement through Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence Immunoassay using the two commercial controls?
2. What is the level of the within-laboratory coefficients of variation (CV^{WL}) of the Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence Immunoassay using the two commercial controls?
3. What are the mean levels of HbA1c measurement using Capillary electrophoresis, High-Performance Liquid Chromatography, and Fluorescence immunoassay, in terms of Total group; Group 1 (HbA1c levels < 6.5 percent); Group 2 (HbA1c levels 6.5 percent - 8.0 percent); and 3.4. Group 3 (HbA1c levels > 8.0 percent)?
4. Is there a significant difference in HbA1c measurement through Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence immunoassay among groups?
5. What is the degree of concordance between Capillary Electrophoresis and High-Performance Liquid Chromatography; and Capillary Electrophoresis and Fluorescence Immunoassay?

6. Is there a significant difference in the slopes of the HbA1c measurements between the standard method, Capillary Electrophoresis, and the High-Performance Liquid Chromatography, and Fluorescence immunoassay methods?

METHODOLOGY

Research Design. This study employed a quantitative approach, descriptive, and comparative research design. According to Creswell (2018), a Quantitative research approach collects and analyzes systematic numerical data to evaluate patterns, relationships, or trends.

Research Locale. The study was conducted in the different laboratories of Cagayan de Oro City, located in the northern part of Mindanao, Philippines, known for its vibrant economy and growing healthcare services. These laboratories extensively modify their laboratory equipment for the advancement of their health services, that is why this locale was chosen.

Selected laboratories in Cagayan de Oro that utilize advanced equipment for HbA1c analysis, including the Bio Rad D-10 Hemoglobin analyzer based on High-Performance Liquid Chromatography, the Sebia Capillarys 2 Flex Piercing for Capillary Electrophoresis, and the Finecare™ HbA1c Rapid Quantitative Test with Fluorescence Immunoassay was included in the study.

Research Samples. The precision analysis in this study utilized commercially available control materials provided by the manufacturers of each measurement system. These control materials included low and high HbA1c controls, in accordance with the CLSI EP15-A3 guidelines. Generally, the low control sample represented HbA1c levels <6.5% (well-controlled), while the high control sample represented HbA1c levels >8.0% (poorly controlled). Each control sample was tested with 25 replicates, performed as five replicates per day over five consecutive days to assess the within-laboratory coefficient of variation (CVWL). These controls were processed according to the manufacturer's specifications to ensure precision and stability, maintaining the integrity of the analysis.

A total of 30 archived whole blood samples were selected from routine HbA1c testing conducted at Capitol University Medical Center in Cagayan de

Oro City. These samples included both inpatient and outpatient specimens, representing a broad range of glycemic profiles. The sample size of 30 specimens adhered to the CLSI EP09-A3 guidelines, which recommended a minimum of 30 specimens for method comparison studies. The samples were grouped based on their HbA1c levels: Group 1 (<6.5%), Group 2 (6.5–8.0%), and Group 3 (>8.0%). The distribution of samples was not evenly divided, reflecting real-world data availability in routine clinical testing, where controlled cases are more common.

For patient samples, inclusion criteria stipulated that only fresh left-over blood samples with reported HbA1c results were included. Samples collected during the morning shift were prioritized to maintain sample integrity and avoid degradation. All samples included were processed within four hours of collection, as HbA1c remained stable within this time under proper storage conditions. K2 EDTA tubes were used for blood collection, as recommended for maintaining sample integrity. Samples that were older or processed outside the morning shift were excluded to prevent inaccuracies.

Statistical Tools. To evaluate the precision, agreement, and variability of HbA1c measurement methods, the study employed a comprehensive set of statistical tools. Descriptive statistics, including the mean and standard deviation, were used to describe the central tendency and dispersion of HbA1c values across Capillary Electrophoresis, High-Performance Liquid Chromatography, and Fluorescence Immunoassay. The within-laboratory coefficient of variation (CVWL) was calculated to assess the precision of each method, where lower CVWL values indicated better reproducibility. The Wilcoxon Signed-Rank Test was utilized to determine statistically significant differences between paired measurements of methods. Method agreement and proportional bias were assessed using Passing-Bablok regression analysis, while Spearman's rank correlation was employed to determine the strength and direction of association between methods without assuming linearity. Bland-Altman plots were generated to visualize the extent of systematic bias and assess agreement between methods. Lastly, Analysis of Covariance (ANCOVA) was performed to compare HbA1c values across methods while adjusting for potential confounders. All statistical analyses were interpreted at an alpha level of 0.05, with p-values below this threshold considered statistically significant.

Ethical Considerations. This investigation was guided and monitored by the UIC-REC to ensure its ethical soundness. Thus, the following ethical

dimensions were validated: social value, informed consent, vulnerability of the research participants, risks, benefits and safety, privacy and confidentiality of the information, justice, transparency, qualification of the researcher, adequacy of facilities, and community involvement.

RESULTS AND DISCUSSION

Level of HbA1C Using Low and High Control

Table 1 presents the HbA1c levels obtained from 25 low and high control samples analyzed using CE, HPLC, and FIA. The CE recorded mean values of 5.80% (low) and 9.04% (high), both within its target range, consistent with Wan Nik et al. (2023), who confirmed CE’s reliability across control levels.

Table 1
Mean Level of HbA1c using the Various Measurement Methods in Two Commercial Controls

Method	Low HbA1c Control	High HbA1c Control
	Mean ± SD	Mean ± SD
High Performance Liquid Chromatography	5.18 ± 0.24	10.16 ± 0.46
Capillary Electrophoresis	5.80 ± 0.10	9.04 ± 0.30
Fluorescence Immunoassay	-	-

Note. N = 25. Target control values: Capillary Electrophoresis: Low Control levels (5.5–6.0 percent); High Control Levels (8.5–9.5 percent). High Performance Liquid Chromatography: Low Control levels (5.0–5.5 percent); High Control Levels (9.5–10.5 percent). Fluorescence immunoassay: no values indicated

The HPLC produced 5.18% and 10.16%, closely aligning with its expected range and supporting the findings of Little et al. (2013) on its clinical precision. In contrast, FIA, which uses fixed internal controls, did not provide specific means but showed stable outputs, aligning with Wang et al. (2020), who noted its utility in environments where predefined calibration suffices.

Both CE and HPLC demonstrated high precision and conformity to target ranges, making them suitable for accurate monitoring of glycemic status. For the FIA, while operationally consistent, may lack the flexibility required for more dynamic quality control needs. These results underscore the need to select

HbA1c methods that align with both clinical goals and laboratory standards.

Within-laboratory Coefficients of Variation for HbA1c Measurements

Table 2 summarizes the within-laboratory coefficients of variation (CV^{WL}) for CE, HPLC, and FIA. The CE showed excellent precision with a CV^{WL} of 1.72% (low) and 3.28% (high), aligning with IFCC and NGSP standards and consistent with findings by Little et al. (2018) and Jeppsson et al. (2018), who noted CE’s low variability across HbA1c levels. The HPLC recorded 4.61% and 4.54%, both under 5% but slightly above the ideal 3% threshold, reflecting Smith et al. (2021) observation of HPLC’s susceptibility to variability, particularly at lower HbA1c concentrations.

Table 2
Within-laboratory Coefficients of Variation (CV^{WL}) of HbA1c Method Measurements in Two Commercial Controls

Method	Low HbA1c Control	High HbA1c Control
	CV^{WL}	CV^{WL}
High Performance Liquid Chromatography	4.61% ^a	4.54% ^a
Capillary Electrophoresis	1.72% ^a	3.28% ^a
Fluorescence Immunoassay	≤10.0% ^a	≤10.0% ^a

Note. IFCC and NGSP criterion: $CV^{WL} < 3$ percent. CLSI general acceptable criterion: < 5 percent: Excellent precision, generally acceptable. 5 - 10 percent: Acceptable for most tests, but further investigation may be needed if precision is critical. > 10 percent: High variability, likely unacceptable. ^a% CV^{WL} measurement is acceptable.

The FIA exhibited CV^{WL} values of ≤10.0%, falling within acceptable limits for routine testing but showing greater variability than CE and HPLC. This aligns with Choi and Han (2024), who acknowledged FIA’s reliability despite higher variation. Overall, CE proved most precise, especially at low HbA1c levels where tight control is critical. While HPLC remains clinically acceptable, further quality control may enhance its precision. Lastly, FIA, although less precise, remains a practical, cost-effective alternative for general monitoring in non-critical settings.

The study’s findings are consistent with the study conducted by Little et al. (2018) and Jeppsson et al. (2018), who demonstrated CE with CV^{WL} values

below 3 percent, showing low variability that is most ideal for routine clinical laboratory use. The values obtained from HPLC are consistent with the study conducted by Smith et al. (2021), although commonly used in clinical laboratories, HPLC is vulnerable to slight variability because of calibration challenges and reagent inconsistencies, particularly at lower HbA1c levels. However, with the CLSI guidelines, this method is still considered acceptable. The FIA results were also highlighted in the study of Choi and Han (2024), who noted that fluorescence based immunoassays tend to show higher variability in their precision due to predetermined values. Still, this method is widely recognized in clinical laboratories, which require less precision.

Levels of HbA1C Using Test Samples

Table 3 presents the mean HbA1c levels across 30 whole blood samples measured by CE, HPLC, and FIA, categorized into three glycemic groups. For the total group, CE and HPLC yielded closely aligned means (7.49% and 7.51%), while FIA was lower at 6.06%. In Group 1 (<6.5%), CE and HPLC again showed similar results (5.91% and 5.85%), with FIA reporting 5.07%. Group 2 (6.5–8.0%) showed comparable patterns across methods. However, in Group 3 (>8.0%), CE and HPLC reported higher averages (10.25% and 10.01%) compared to FIA (9.24%).

Table 3
Mean Levels of HbA1c Measurement among Groups

Method	CE	FIA	HPLC
	Mean ± SD	Mean ± SD	Mean ± SD
Total group	7.49 ± 2.05	6.06 ± 1.76	7.51 ± 2.00
Group 1a	5.91 ± 0.39	5.07 ± 0.69	5.85 ± 0.43
Group 2b	7.17 ± 0.41	6.88 ± 0.31	7.07 ± 0.41
Group 3c	10.25 ± 1.96	9.24 ± 1.29	10.01 ± 1.76

Note. N = 30. CE – Capillary electrophoresis, FIA – Fluorescence Immunoassay, HPLC – High performance Liquid Chromatography.

^aGroup 1—HbA1c levels<6.5 percent

^bGroup 2—HbA1c levels 6.5%-8.0%

^cGroup 3—HbA1c levels> 8.0%

These results reinforce the findings of Jeppsson et al. (2018), confirming CE and HPLC’s precision across all glycemic levels. For the results of FIA, while consistent in mid-ranges, showed underestimation at higher levels, echoing limitations noted by Wang et al. (2020) and Vesper & Miller (2019). Thus, CE

and HPLC remain reliable methods for HbA1c testing, while FIA may require careful interpretation, particularly at elevated glucose levels.

Test of the differences of HbA1c Methods

Table 4 presents the Wilcoxon Signed-Rank Test results comparing HbA1c measurements from CE, HPLC, and FIA across glycemic groups. The CE served as the reference method.

No significant differences were found between CE and HPLC in the total group ($p=0.710$) or within Group 1 ($<6.5\%$, $p=0.633$), Group 2 ($6.5\text{--}8.0\%$, $p=0.346$), and Group 3 ($>8.0\%$, $p=0.340$), confirming their consistent performance across all HbA1c ranges. In contrast, CE and FIA showed significant differences across all groups ($p\text{-values} < 0.05$), indicating variability in FIA measurements at both low and high HbA1c levels.

Table 4
Significant Differences in HbA1c Measurements among Groups

Groups	Methods Compared	Z	p	Remarks
Total group	CE-HPLC	-0.372	0.710	Not significant
	CE-FIA	-4.78	<0.001	Significant
Group 1 ^a	CE-HPLC	-0.478	0.633	Not significant
	CE-FIA	-3.062	0.002	Significant
Group 2 ^b	CE-HPLC	-0.942	0.346	Not significant
	CE-FIA	-2.803	0.005	Significant
Group 3 ^c	CE-HPLC	-0.954	0.340	Not significant
	CE-FIA	-2.2521	0.012	Significant

Note. CE – Capillary electrophoresis, HPLC – High-performance Liquid Chromatography, FIA – Fluorescence Immunoassay.

^aGroup 1—HbA1c levels<6.5%

^bGroup 2—HbA1c levels 6.5%-8.0%

^cGroup 3—HbA1c levels> 8.0%

These findings support Jeppsson et al. (2018), who affirmed the precision of CE and HPLC, and align with Wang et al. (2020) and Vesper & Miller (2019), who reported discrepancies in FIA results due to underestimations and calibration issues. Overall, CE and HPLC remain robust and reliable for HbA1c analysis, while FIA requires cautious application in clinical settings demanding high accuracy.

Concordance Analysis of HbA1c Measurements

Tables 5.1 and 5.2 and Figures 3 and 4 summarize the concordance analysis between HPLC, and FIA using Passing-Bablok regression, Spearman correlation, and Bland-Altman plots.

Table 5.1
Passing-Bablok Regression Analysis and Spearman Rank Correlation Study of

Method	Comparison	Intercept (95% CI)	Slope (95% CI)	Correlation coefficient (95% CI)	p
Total	CE-HPLC	-0.100 (-0.51 – – 0.27)	1.00 (0.96 – 1.06)	0.986 (0.97 – 0.99)	<0.001
	CE-FIA	0.705 (-0.93 – 2.20)	1.10 (0.85 – 1.34)	0.875 (0.77 – 0.94)	<0.001
Group 1	CE-HPLC	0.63 (-2.33 – 3.65)	0.91 (0.38 – 1.40)	0.785 (0.35 – 0.93)	0.002
	CE-FIA	4.34 (0.95 – 6.21)	0.32 (-0.07 – 1.00)	0.057 (-0.53 – 0.61)	0.860
Group 2	CE-HPLC	1.43 (-1.04 – 2.43)	0.80 (0.67 – 1.12)	0.922 (0.67 – 0.98)	<0.001
	CE-FIA	4.88 (-10.85 – 7.66)	0.39 (-0.09 – 3.00)	0.297 (-0.42 – 0.77)	0.404
Group 3	CE-HPLC	-0.22 (-4.12 – 3.17)	1.03 (0.67 – 1.41)	0.960 (0.76 – 0.99)	<0.001
	CE-FIA	1.16 (-3.60 – 8.41)	1.04 (0.13 – 1.65)	0.810 (0.19 – 0.96)	0.015

HbA1c Measurements using Various Methods
Note. CE-Capillary electrophoresis, FIA-Fluorescence Immunoassay, HPLC-High-performance Liquid Chromatography.
^aGroup 1—HbA1c levels<6.5%
^bGroup 2—HbA1c levels 6.5%-8.0%
^cGroup 3—HbA1c levels> 8.0%
**significant at p <0.001
* significant at p <0.05

The CE and HPLC showed excellent agreement across all HbA1c levels. In the total group, regression analysis produced a slope of 1.00 and intercept of -0.10, with a strong correlation (r = 0.986, p < 0.001), indicating near-identical performance. The Bland-Altman analysis showed negligible bias (0.02), further confirming their interchangeability. These findings are consistent with Jeppsson et al. (2018) and Vesper et al. (2019), who emphasized the reliability and alignment of these two methods.

In contrast, CE and FIA revealed variability. While the total group correlation remained moderate ($r = 0.875$), the regression slope (1.10) and intercept (0.705) indicated proportional bias. Bland-Altman plots showed a significant negative bias (-1.42), consistent with Wang et al. (2020) and Zhao et al. (2019), who reported underestimation trends in FIA, especially at low and high HbA1c levels. Group-specific results echoed this inconsistency, with FIA displaying weak correlations and pronounced bias in Groups 1 and 3.

The combined use of regression and bias analyses revealed differences in method agreement: regression highlighted proportional relationships, while Bland-Altman exposed absolute discrepancies. As recommended by ISO 15189 and CLSI EP09, both tools are crucial for method evaluation. Overall, CE and HPLC demonstrated strong concordance and minimal variability, making them dependable for clinical use. For the FIA, while convenient, requires calibration and should be cautiously interpreted in precision-critical settings.

The findings of the study align with the results of Jeppsson et al. (2018), who reported that CE and HPLC produce high precision and alignment across all HbA1c ranges, suggesting a good method for routine clinical usage. The discrepancies of results with FIA across all groups are consistent with the findings of Wang et al. (2020), who highlighted that due to fluorescence-based detection there is variability of the performance when measuring HbA1c levels especially at lower HbA1c levels. As emphasized by the study of Vesper et al. (2019), there is a need for the calibration of FIA to be enhanced to align with established methods like CE and HPLC.

Table 5.2
Bland-Altman analysis of HbA1c Measurements using Various Methods

Groups	Methods Compared	Bias	Standard error	95% Confidence Interval	p
Total	CE-HPLC	0.02	0.35	-0.66 – 0.69	0.754
	CE-FIA	-1.42	0.99	-3.37 – 0.52	<0.001**
Group 1 ^a	CE-HPLC	0.00	0.28	-0.18 – 0.18	1.00
	CE-FIA	-1.08	0.77	-1.57 – -0.59	0.001**
Group 2 ^b	CE-HPLC	0.06	0.18	-0.7 – 0.19	0.329
	CE-FIA	-1.25	0.82	-1.84 – -0.66	0.001**
Group 3 ^c	CE-HPLC	0.00	0.57	-0.48 – 0.48	1.00
	CE-FIA	-2.15	1.20	-3.15 – -1.15	0.001**

Note. CE – Capillary electrophoresis, HPLC – High-performance Liquid Chromatography, FIA – Fluorescence Immunoassay

^aGroup 1—HbA1c levels<6.5%^bGroup 2—HbA1c levels 6.5%-8.0%^cGroup 3—HbA1c levels> 8.0%**significant at $p < 0.001$

The findings are consistent with those reported by Jeppsson et al. (2018) in the Bland-Altman analysis, which highlights a strong agreement between CE and HPLC, which is recommended for routine clinical HbA1c testing. The significant biases observed with FIA aligns with the observations of Wang et al. (2020), who noted underestimation of HbA1c levels, particularly in the lower and higher ranges. The observation of Vesper et al. (2019) suggests the need for calibration and control in FIA to improve its reliability, which is shown by the significant biases observed in this study.

Comparison of Slope Estimates

Table 6 compares slope estimates among CE, HPLC, and FIA to assess linearity. No significant slope difference was observed between CE and HPLC (mean difference = 0.020, $p = 0.999$), confirming strong linear alignment and supporting HPLC's reliability as an alternative to CE.

In contrast, significant slope differences were found between CE and FIA (mean difference = 1.423, $p = 0.015$) and between HPLC and FIA (mean difference = 1.443, $p = 0.014$), indicating greater variability and less consistent linearity in FIA measurements.

The main effects analysis revealed significant differences among methods ($F = 5.447$, $p < 0.001$, $\eta^2 = 0.118$) and HbA1c groups ($F = 19.233$, $p < 0.001$, $\eta^2 = 0.622$), while interaction effects were minimal ($F = 0.849$, $p = 0.498$). These findings support prior work by Jeppsson et al. (2018), which demonstrated strong linearity in CE and HPLC, and align with Wang et al. (2020), who reported slope variability in FIA.

Overall, CE and HPLC showed robust linear performance, reinforcing their use in clinical HbA1c testing. The variability in FIA slope estimates suggests the need for calibration and method refinement to ensure reliability in clinical and laboratory applications.

Table 6
Significant Differences in the Slopes of the HbA1c Measurements using Various Methods

Methods Compared	Mean difference	t	p	Interpretation
CE-HPLC	0.020	0.040	0.999	Not significant
CE-FIA	1.423	2.838	0.015	Significant
HPLC-FIA	1.443	2.878	0.014	Significant

Note. CE – Capillary electrophoresis, HPLC – High-performance Liquid Chromatography, FIA – Fluorescence Immunoassay
Method main effects – $F = 5.447$, $p = <0.001$, $\eta^2 = 0.118$
Group main effects – $F = 19.233$, $p = <0.001$, $\eta^2 = 0.622$
Methods and Group interaction effects – $F = 0.849$, $p = 0.498$, $\eta^2 = 0.010$

These findings were consistent with the study of Jeppsson et al. (2018), who demonstrated a strong linearity of CE and HPLC in HbA1c measurement. In contrast, in the study of Wang et al. (2020), a similar report of discrepancies in FIA slope estimates particularly when compared to standard methods like CE. The significant differences between FIA and the other two methods observed in this study align with these reports, further emphasizing the need for Fluorescence Immunoassay calibration to achieve comparable linearity.

The results highlight the accuracy and reliability of CE and HPLC because they maintain consistent slope estimates, supporting their reliability for routine clinical usage. The FIA shows significant slope deviations, which affect the utility of the method in producing accurate and reliable HbA1c measurements. This study further highlights the importance of method concordance when evaluating variability and capability of the test methods used in routine clinical use.

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