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ORIGINAL ARTICLE

Comparative evaluation of HbA1c in point-of-care testing (POCT) devices: enzymatic and immunoassay methods compared to ion-exchange high-performance liquid chromatography (IE-HPLC)

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ABSTRACT

Aim To evaluate 3 point-of-care testing (POCT) devices in testing HbA1c: the Simplex TAS™101 (Tascom Co. Ltd., South Korea), which utilizes an enzymatic method, and two immunoassay-based devices, the Zybio Q8 Pro and Zybio EXR 110 (Zybio Inc., China).
Methods A total of 40 whole blood samples with EDTA anticoagulant were collected from patients undergoing HbA1c testing at an outpatient department. The samples were analysed using 3 POCT devices and one reference method.
Results Passing-Bablok regression revealed no significant fixed or proportional bias. Strong correlations with the gold standard were observed for Simplex TAS™101 (r=0.944), Zybio Q8 Pro (r=0.910), and Zybio EXR 110 (r=0.839), all with p<0.001. Bland-Altman analysis confirmed that the majority of measurements fell within an acceptable clinical range**.**
Conclusion This study demonstrated that the three POCT devices employing enzymatic and immunoassay methods served as reliable alternatives to ion exchange-high-performance liquid chromatography (IE-HPLC) for HbA1c measurement in clinical practice.
Keywords: diabetes, laboratory, point-of care testing

INTRODUCTION

Glycated haemoglobin (HbA1c) is widely recognized as a critical biomarker for assessing long-term glycaemic control in patients with diabetes (1). This parameter provides an accurate reflection of average blood glucose levels over the preceding two to three months, making it a reliable indicator for evaluating the risk of diabetes-related complications (1,2). In addition to its role in monitoring, HbA1c is also utilized as a diagnostic criterion for diabetes (2). Both the American Diabetes Association (ADA) (3) and the World Health Organization (WHO) (4) have approved HbA1c testing as a standard diagnostic tool, with a recommended threshold of 6.5% (5).

HbA1c levels are commonly measured through the collection of a venous blood sample, which is subsequently sent to a laboratory for analysis. While this method is widely used, it is often associated with considerable time and cost implications (6). Point-of-Care Testing (POCT) HbA1c devices allow testing to be conducted in diverse settings, such as doctors' offices, outpatient clinics, pharmacies, and nursing facilities. These devices require smaller sample volume and primarily utilize capillary blood obtained through a finger stick, though certain models also accommodate venous blood samples (7). POCT technology has significantly advanced clinical laboratory practices by providing reliable and user-friendly equipment with demonstrated clinical benefits. Its accessibility and ease of use, supported by low-maintenance analysers and visually interpretable kits, offer an effective solution for conducting laboratory tests directly at or near the patient's location (8). The adoption of POCT for HbA1c has been linked to shorter turnaround times, improved glycaemic control in patients, timely delivery of test results with corresponding treatment adjustments, enhanced decision-making processes, reduced need for multiple patient visits, and improved communication between patients and medical professionals (6).

High-performance liquid chromatography (HPLC) has been the primary method for HbA1c analysis, offering precise measurements with high resolution. In recent years, alternative methods such as immunoassays and enzymatic assays have

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been introduced, enabling HbA1c measurement through a variety of methodologies (9). Studies on method evaluation and comparison indicate varying performance among different HbA1c POCT devices. Ensuring accurate HbA1c measurements and reliable results across diverse clinical settings is essential in the current era of POCT (10).

The aim of this study was to evaluate the performance of POCT devices utilizing enzymatic and immunoassay methods in comparison with IE-HPLC, the reference method used in the central laboratory settings.

PATIENT AND METHODS

Patients and study design

This study was conducted from August 2025 to September 2025 at the Central Laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. A total of 40 fresh blood samples were collected from patients undergoing HbA1c testing at the outpatient department. Exclusion criteria included patients <18 years of age, pregnant women, and patients with anaemia. For each patient, 5 mL of whole blood was drawn using EDTA as an anticoagulant.

A total of 40 samples were analysed using four devices, including three point-of-care testing (POCT) devices such as Simplex TAS™101 (Tascom Co. Ltd., South Korea), Zybio Q8 Pro (Zybio Inc., China), and Zybio EXR 110 (Zybio Inc., China), and a gold-standard examination using BioRad D-10 (Bio-Rad Laboratories, USA). We chose 40 samples, as it is the minimum samples required for validation study (11). The results from the POCT devices were systematically compared with those obtained from the gold-standard device for evaluation.

A written informed consent was obtained from each patient or their representative after a thorough explanation. This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya (approval letter number: 0744/KEPK/VIII/2023).

Methods

Three HbA1c POCT devices were evaluated: the Simplex TAS™101 utilizes an enzymatic method, and two immunoassay-based devices, the Zybio Q8 Pro and Zybio EXR 110. The enzymatic HbA1c assay is based on the enzymatic digestion of lysed whole blood samples by proteases, which break down haemoglobin beta chains into amino acids, including glycated dipeptides. These glycated dipeptides act as substrates for fructosyl peptide oxidase (FPOX), an enzyme that specifically cleaves N-terminal valines, generating hydrogen peroxide as a byproduct. The resulting hydrogen peroxide is subsequently quantified through a reaction catalysed by horseradish peroxidase (POD) in the presence of an appropriate chromogenic substrate. The immunoassay-based methods used immunofluorescence chromatography and a double-antibody sandwich method for rapid, quantitative detection of HbA1c in whole blood. The test line, coated with a mouse anti-human HbA1c monoclonal antibody, captures an antigen-antibody complex formed between sample HbA1c, Hb, and a fluorescently labelled antibody. The complex migrates via capillary action, producing a fluorescent band. An immunofluorescence analyser quantifies HbA1c by analysing the fluorescence signal (12).

The BioRad D-10 was utilized as a reference method for HbA1c measurement. This device operates on the principle of ion-exchange high-performance liquid chromatography (IE-HPLC) using whole blood samples anticoagulated with ethylenediaminetetraacetic acid (EDTA). The method enables the separation of HbA1c and HbA0 based on differences in their isoelectric points, leveraging variations in ionic interactions. The separated haemoglobin fractions are subsequently passed through a flow cell equipped with a photometric filter and measured based on absorbance changes at a wavelength of 415 nm. The HPLC method is widely recognized for its ability to eliminate labile components, such as pre-A1c or Schiff base. These components originate from the initial phase of haemoglobin (HbA) glycation, a rapid and reversible process that is highly dependent on plasma glucose concentration (13).

Two concentrations of Quality Control (QC) materials (Level 1 and Level 2) were used in this study. Precision verification was conducted by analysing five replicates of each QC level daily over a period of five days across the three POCT devices. Imprecision was assessed by calculating the total %CV for Within-Laboratory conditions (14).

Statistical analysis

The normality of data distribution was assessed using the Shapiro-Wilk test. Passing-Bablok regression analysis was applied to evaluate the linear relationship and potential systematic bias between the measurement methods. Spearman's rank correlation test (r) was conducted to determine the strength and direction of the association between the two methods, with statistical significance set at $p<0.05$. Finally, method agreement was assessed using Bland-Altman analysis to define the limits of agreement for HbA1c level variations across different measurement devices.

RESULTS

This study analysed data from 40 patient samples, consisting of 18 males and 22 females aged between 32 and 69 years. HbA1c levels were measured in all participants (Table 1).

Table 1. Characteristics of HbA1c from 40 patients for each device

Device	Median of HbA1c (min – max)
BioRad D-10	6.40% (4.70–12.00)
Simplex TAS™101	6.20% (4.50–10.70)
Zybio Q8 Pro	6.89% (5.00–12.37)
Zybio EXR 110	6.54% (5.00–11.25)

Precision testing with 25 repetitions of level 1 and Level 2 controls demonstrated that the lowest %CV values were observed for the Simplex TAS™101 (L1=2.8%; L2=1.4%), whereas the highest %CV values were measured for the Zybio Q8 Pro (L1=3.26%; L2=4.8%) (Table 2).

The correlation analysis results of HbA1c measurements from 40 patient samples were assessed using four different devices (Table 3).

The statistical analysis comparing SimplexTAS™ and BioRad D-10 (N=40) demonstrated a strong agreement and correlation between the two methods. The Passing–Bablok regression analysis between Simplex TAS™101 and BioRad D-10 (N=40) yielded an intercept of −0.1326 (95% CI: −0.6252-0.3588) and a slope of 0.9565 (95% CI: 0.8824-1.028), indicating no

Table 2. Total precision testing result of HbA1c for each device

Device	Control level 1			Control Level 2		
	Mean	SD	%CV	Mean	SD	%CV
Simplex TAS™101	5.95	0.16	2.8	8.7	0.12	1.4
Zybio EXR 110	5.28	0.14	2.8	9.01	0.43	4.7
Zybio Q8 Pro	5.32	0.17	3.2	9.07	0.43	4.8
BioRad D-10	5.32	0.13	2.4	10.32	0.16	1.6

SD, standard deviation; CV, coefficient of variation

significant fixed or proportional bias (Table 3). Spearman's correlation analysis showed a very strong positive correlation (r=0.944; p<0.001), confirming the consistency between the two methods (Figure 1A). Bland-Altman analysis revealed a mean bias of -0.45, indicating that SimplexTAS™ tended to measure slightly lower HbA1c levels than BioRad D-10. The 95% limits of agreement ranged from −1.29 to 0.38, with the majority of data points falling within these limits (Figure 2A). The statistical analysis comparing Zybio EXR 110 and BioRad

Table 3. Correlation analysis of HbA1c level between different point-of-care testing (POCT) SimplexTASTM101, Zybio EXR 110, and Zybio Q8 Pro with BioRad D-10

POCT device	r Spearman	Slope	Intercept	Mean bias	p value
Simplex TAS™101 vs. BioRad D-10	0.944	0.9565	–0.1326	–0.45	<0.001
Zybio EXR 110 vs. BioRad D-10	0.839	0.9515	0.1561	–0.04	<0.001
Zybio Q8 Pro vs. BioRad D-10	0.910	1.011	0.059	0.2	<0.001

r Spearman, correlation coefficient; Slope, a slope of 1 indicates perfect association; Intercept, a point where a line crosses y-axis; Mean bias, tendency of underestimate or overestimate.

D-10 (N=40) demonstrated a strong correlation and overall agreement between the two methods. The Passing–Bablok regression analysis between Zybio EXR 110 and BioRad D-10 (N=40) yielded an intercept of 0.1561 (95% CI: −0.6624-0.9975) and a slope of 0.9515 (95% CI: 0.8211-1.072), in-

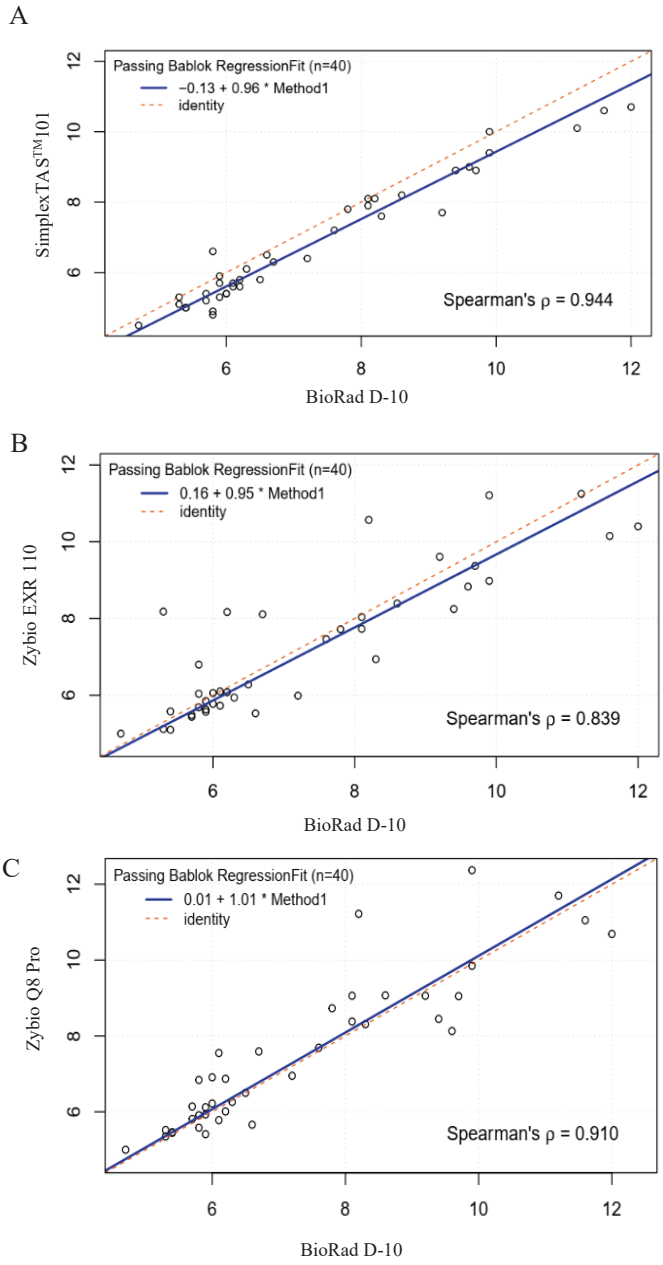

Figure 1. Scatterplot of HbA1c level for: A) the SimplexTASTM101 and BioRad D-10; B) Zybio EXR 110 and and BioRad D-10; C) Zybio Q8 Pro and BioRad D-10

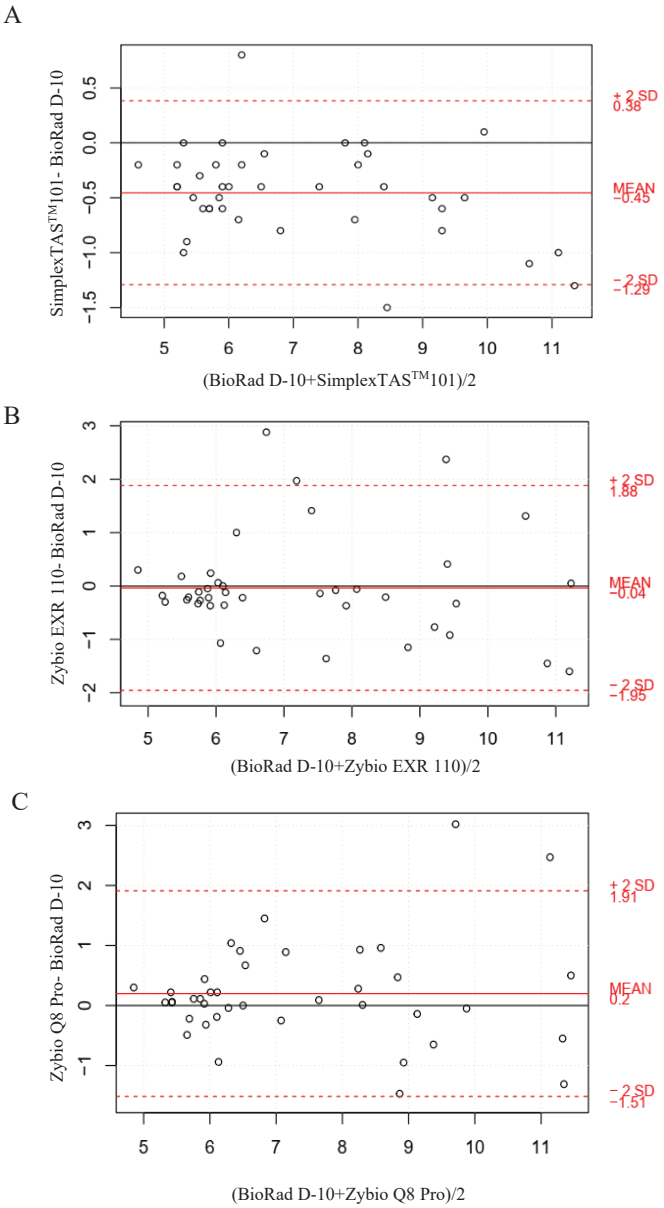

Figure 2. Blant Altman analysis of HbA1c between: A) the SimplexTASTM101 and BioRad D-10; B) Zybio EXR 110 and and BioRad D-10; C) Zybio Q8 Pro and BioRad D-10

dicating no significant fixed or proportional bias (Table 3). Spearman's correlation analysis revealed a strong positive correlation (r=0.839; p<0.001), confirming a consistent relationship between the two methods despite some variability (Figure 1B). Bland–Altman analysis revealed a mean bias of -0.04, suggesting that Zybio EXR 110 reported slightly lower HbA1c values compared to BioRad D-10. The 95% limits of agreement ranged from −1.95 to 1.88, with most data points falling within this interval, suggesting acceptable agreement between the two methods (Figure 2B).

The statistical analysis comparing Zybio Q8 Pro and BioRad D-10 (N=40) demonstrated a strong correlation and overall agreement between the two methods. The Passing–Bablok regression analysis between Zybio Q8 Pro and BioRad D-10 (N=40) yielded an intercept of 0.0059 (95% CI: −1.078-0.784) and a slope of 1.011 (95% CI: 0.8952-1.183), indicating no significant fixed or proportional bias (Table 3). Spearman's correlation analysis revealed a very strong positive correlation (r=0.910; p<0.001), confirming a high level of agreement between the two methods (Figure 1C). Bland–Altman analysis revealed a mean bias of 0.20, indicating that Zybio Q8 Pro tended to slightly overestimate HbA1c values compared to the BioRad D-10. The 95% limits of agreement ranged from −1.51 to 1.91, with most data points falling within this interval, suggesting that the level of disagreement remains within an acceptable clinical range (Figure 2C).

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia, which, if left uncontrolled, can lead to severe long-term degenerative complications. Therefore, the diagnosis, management, and monitoring of diabetic patients require comprehensive laboratory testing for assessing glycaemic control and guiding appropriate therapeutic interventions (15–17). HbA1c provides a cumulative representation of blood glucose levels over the preceding two to three months, making it a more reliable indicator of chronic hyperglycaemia. HbA1c measurement does not require fasting conditions or specific timing for blood sample collection, thereby enhancing its practicality and convenience in routine clinical monitoring and diabetes management (18)

The three devices used in this study are widely available in Asia, but they have not yet undergone validation, making this study the first to validate all of these devices. POCT-based HbA1c analysers are now widely integrated into routine clinical practice, offering rapid and reliable results while minimizing the need for centralized laboratory testing. These devices have proven particularly beneficial in resource-limited settings or situations where conventional laboratory assessments are too time-consuming. For instance, paediatric patients, individuals with poorly controlled diabetes, and those requiring frequent monitoring can greatly benefit from near-patient HbA1c testing. Furthermore, POCT devices enable real-time decision-making by healthcare providers, allowing for immediate adjustments in diabetes treatment regimens (19).

A key challenge in HbA1c measurement lies in the standardization and reliability of different analytical methods. Variability in measurement techniques can lead to inconsistent results, potentially affecting clinical decision-making. Therefore, numerous studies have been conducted to evaluate the analytical performance of HbA1c assays, comparing them with established reference methods to ensure accuracy, precision, and reproducibility (14,20). In this study, Simplex TAS™ 101 exhibited the lowest total coefficient of variation (%CV) among the three evaluated devices, indicating superior precision. The comparative analysis of SimplexTAS™101, Zybio EXR 110, and Zybio Q8 Pro against BioRad D-10 demonstrated a very strong correlation and overall agreement, with slight variations in bias. SimplexTAS™101 showed the highest association with BioRad D-10, followed by Zybio Q8 Pro and Zybio EXR 110. The Spearman test showed a very strong correlation for all three devices, indicating that the HbA1c results from these devices can be considered a reliable alternative (21).

Passing-Bablok regression analysis indicated no significant fixed or proportional bias, as all confidence intervals encompassed zero and the slopes were close to one. Bland-Altman analysis further confirmed that while minor differences existed between the methods, the majority of measurements fell within an acceptable clinical range. Despite these variations, all three devices demonstrated high comparability with BioRad D-10, making them viable alternatives for HbA1c measurement. However, the observed biases should be taken into account when interpreting clinical results.

Haemoglobin variants may interfere with HbA1c measurements due to various factors. The reference method meets the requirements for high throughput, accuracy, and reliability in HbA1c testing. It is also capable of detecting haemoglobin variants, which can be considered both an advantage and a disadvantage (22). Unlike reference methods, our POCT devices utilized chemical approaches such as enzymatic and immunoassay techniques, which are easily implemented on standard chemistry analysers. These methods do not detect haemoglobin variants and do not interfere with HbA1c measurements, offering a more streamlined approach but at the cost of lacking variant detection capabilities (23).

The limitations of this study include a relatively small sample size although it meets the criteria of the Clinical Laboratory Improvement Amendments (CLIA), the fact that it does not take into account potential interference factors, such as hemoglobinopathies that may be present in certain populations, and last, the study was conducted at only one study centre. Further validation studies involving larger, multicentre, and more diverse sample populations are recommended to refine clinical thresholds and confirm the generalizability of these findings.

In conclusion, enzymatic and immunoassay methods, which strongly correlate with HPLC, enhance accuracy and reliability in clinical practice. This study confirms the precision of the three POCT devices, demonstrating their strong correlation with reference methods. POCT-based HbA1c analysers are valuable for diabetes management, particularly in settings requiring immediate clinical decisions.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, validation, formal analysis, data curation: WRA, YP, and FRM; Software: WRA and FRM; Investigation, resources, visualization, supervision: YP. Data curation, writing, project administration: WRA and YP; Funding acquisition: YP and FRM.

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TRANSPARENCY DECLARATION

Although this study was supported by several providers, the sponsors had no influence on the study design, data collection, analysis, interpretation, or the preparation of this manuscript.

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