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Received 6 Oct 2025
Revised 3 Nov 2025
Accepted 3 Nov 2025
Published Online 6 Nov 2025

Evaluation of the HbA1c Analytical Performance on the Bio-Rad D-10 Analyzer Using Sigma Metrics at the Clinical Diagnostic Laboratory, USM Medical Centre Bertam

Abstract **–** Glycated haemoglobin (HbA1c) is a key biomarker for long-term glycaemic control in diabetes management, necessitating high analytical accuracy and precision. This study evaluated the performance of HbA1c assay on the Bio-Rad D-10 analyzer, which employs high-performance liquid chromatography (HPLC) method, using Sigma metrics at the Clinical Diagnostic Laboratory (CDL), USM Medical Centre Bertam (PPUSMB). A retrospective analysis of two-level internal quality control (IQC) data from February to August 2025 was conducted. Imprecision was calculated and expressed as coefficient of variation (CV%), bias was determined from Unity Interlaboratory Program data, and Sigma scores were calculated according to CLIA 2024 guideline. Results revealed variable performance at QC Level 1, with Sigma scores ranging from 3.30 to 9.01, indicating marginal to world-class quality. The performance variability was attributed to higher bias and imprecision at lower analyte concentrations. In contrast, QC Level 2 consistently demonstrated excellent to world-class performance, with Sigma scores between 5.63 and 14.70, supported by low bias (0.20–0.79%) and CV% (0.50–0.93%). In conclusion, the Bio-Rad D-10 analyzer provides robust analytical performance, particularly at higher HbA1c concentrations. However, lower concentration levels require closer monitoring and strengthen quality control monitoring strategies to maintain results' reliability. Sigma metrics offer a structured framework for evaluating method performance but should be complemented by additional quality indicators for comprehensive assessment.

Keywords **–** HbA1c, Bio-Rad D-10, high-performance liquid chromatography, Six Sigma, analytical performance, quality control

1 INTRODUCTION

Type 2 diabetes mellitus (T2DM) has become a global epidemic, with prevalence continuing to rise at an alarming rate (1). The International Diabetes Federation (IDF) estimates that one in nine adults worldwide is living with diabetes, a figure that is expected to increase substantially in the coming decades, resulting in immense healthcare and socioeconomic burdens (2). The disease is associated with serious long-term complications including cardiovascular disease, nephropathy, neuropathy, and retinopathy, which significantly reduce quality of life and contribute to premature mortality (3).

Among the biomarkers available for monitoring glycaemic control, glycated haemoglobin A1c (HbA1c) has become the cornerstone in both clinical practice and research. HbA1c reflects the mean blood glucose concentration over the previous 8 to 12 weeks, thereby offering a more comprehensive picture of long-term glycaemic status compared to fasting plasma glucose or oral glucose tolerance testing (4). International guidelines now recommend HbA1c as a diagnostic tool for diabetes and as the preferred biomarker for treatment monitoring due to its ability to predict the risk of diabetes-related complications (5).

The accuracy and precision of HbA1c testing are paramount to ensuring reliable clinical decision-making. High-performance liquid chromatography (HPLC) is widely regarded as the gold standard for HbA1c measurement because of its superior ability to separate haemoglobin fractions and minimize analytical interference. The Bio-Rad D-10 Haemoglobin Testing System, based on reverse-phase cation-exchange HPLC, has emerged as one of the most widely adopted automated platforms for HbA1c measurement due to its integration of sample preparation, separation, and quantification within a single module (6).

Recent comparative studies reinforce the importance of HPLC-based methods. For example, Zechmeister et al. (2023) demonstrated that while enzymatic assays can achieve excellent precision and strong correlation with HPLC results, HPLC continues to provide benchmark reliability (7). Similarly, evaluations comparing HPLC with immunoassays, enzymatic methods, and capillary electrophoresis have shown high levels of agreement but also

highlighted small biases that may impact clinical interpretation, particularly in patients near diagnostic cutoffs. In cases involving haemoglobin variants, capillary electrophoresis has been shown to provide complementary benefits by detecting variant interferences, thus supporting more accurate HbA1c assessment (8).

Alongside technological advancements in analytical methods, laboratories are increasingly adopting structured quality management frameworks to ensure reliable results. Six Sigma methodology, adapted from industrial process control, offers an objective performance assessment tool by integrating bias, imprecision (expressed as CV), and total allowable error (TEa). A Sigma score of ≥6 is considered "world-class," while values below 3 indicate unacceptable performance requiring corrective actions (9). Its application in laboratory medicine has provided valuable insights into method reliability, with studies reporting world-class Sigma performance for HbA1c testing on the Bio-Rad D-10 analyzer (10).

Local evaluation of analyzer performance is essential to ensure the reliability of laboratory results under real-world conditions. While manufacturers provide validation data, these are often generated in controlled settings that may not reflect the unique characteristics of local populations, laboratory environments, or operator practices. Factors such as haemoglobin variants, anaemia prevalence, environmental conditions, and sample handling can significantly influence analyzer performance, particularly for tests like HbA1c that are critical in diagnosing and monitoring diabetes. In addition, conducting multicenter validation studies across different populations is crucial. For example, a study evaluating sigma metric of an HbA1c analyzer reported excellent reproducibility but also identified a negative bias in samples from anemic patients, underscoring that population-specific factors can influence the accuracy of HbA1c results (10). Similarly, a Malaysian study comparing HPLC with capillary electrophoresis reported that haemoglobin variants, common in the local population, affected HbA1c measurement when values exceeded the analyzer's linearity range (11). These findings highlight that local performance evaluations are not only critical for verifying manufacturer claims but also for optimizing quality control strategies, meeting accreditation requirements, and, most importantly, safeguarding patient care by ensuring accurate results at clinically significant thresholds.

Given the critical role of HbA1c and the requirement of a stringent analytical performance, this study evaluates the HbA1c assay performance on Bio-Rad D-10 analyzer using Sigma metrics. By quantifying performance in terms of accuracy, precision, and error tolerance, the study provides evidence to support best practice in laboratory medicine and contributes to improving the reliability of diabetes management worldwide.

2 MATERIALS AND METHODS

2.1 Study Design

This study was conducted in the Integrated Unit (Chemical Pathology), Clinical Diagnostic Laboratory (CDL), USM Medical Centre Bertam (PPUSMB), an MS ISO 15189 accredited diagnostic facility offering comprehensive laboratory services including haematology, biochemistry and immunology. Internal quality control (IQC) data for HbA1C were retrospectively reviewed over a seven-month period from February to August 2025. The dataset was derived from the analysis of two levels of HbA1c QC materials (Bio-Rad Lyphocheck Diabetes Control Level 1 and Level 2) using Bio-Rad D-10 analyzer (Bio-Rad Laboratories, Inc., California, USA), which operates on the principle of reverse-phase cation-exchange HPLC. The Lyphochek Diabetes Control is an assayed, lyophilized human whole blood–based control designed to monitor the accuracy and precision of HbA1c measurements in diabetes-related assays.

Prior to analysis, QC materials of both levels were prepared and handled strictly according to the manufacturer's instructions. Each vial was mixed thoroughly before use, and repeated freeze–thaw cycles of opened vials were avoided to maintain stability.

The laboratory means and standard deviation (SD) were established from IQC results for each control level. Acceptability criteria were defined as the mean ± 2SD, in accordance with CLSI EP5-A2 guidelines, and were used for routine monitoring of assay performance. Coefficient variations of percentage (CV%) and bias were calculated and used as parameters to determine Sigma score for each QC level (12). The analytical performance of HbA1c assay was then evaluated for each month based on the Sigma scores, from where the optimal QC strategy was derived from.

2.2 Coefficient of Variation

Coefficient of variation (CV) is a statistical measure of imprecision, expressed as percentage (CV%). The degree of analytical precision was determined from monthly IQC data using the following formula:

CV (%) = [Standard Deviation (SD) / Laboratory Mean] × 100

where SD and Laboratory Mean were calculated from IQC results for each control level.

2.3 Bias

Bias represents the systematic difference between the measured value and the true value and is inversely related to the concept of trueness. In this study, monthly bias was derived from the percentage deviation of peer-group data obtained through the Unity Interlaboratory Program (UNITY). Bias was calculated using the following formula:

Bias (%) = [(Peer Group Mean − Laboratory Mean) / (Peer Group Mean)] × 100

where Peer Group Mean refers to the mean value of laboratories using the same instrument and analytical method, and Laboratory Mean refers to the mean value obtained in our laboratory.

2.4 Total Allowable Error (TEa)

Total Allowable Error (TEa) is a performance model that represents the maximum permissible error in a test result and integrates both imprecision and bias (trueness) of a method to estimate its overall impact on test results. For this study, the TEa was defined as 8% according to the Clinical Laboratory Improvement Amendments (CLIA) 2024 Proficiency Testing Criteria. The CLIA 2024 update was introduced through Centers for Medicare & Medicaid Services (CMS) final rules and guidance released in late 2023 and throughout 2024, with certain provisions extending into 2025.

2.5 Sigma Metrics Calculation

Sigma scores were calculated for each month at both QC levels to assess the overall analytical performance of the HbA1c assay. The calculation incorporated imprecision (CV%), bias (%), and TEa using the standard formula:

Sigma (σ) = [TEa − Bias (%)] / CV (%)

2.6 Interpretation of Analytical Performance

Analytical methods were grouped into six categories of performance based on Sigma scale as presented in Table I. A Sigma value of six or higher reflects world-class quality, requiring minimal QC oversight. Values between five and six indicate excellent performance, while those between four and five are considered good. Scores between three and four are acceptable but call for stricter QC procedures. When Sigma values fall between two and three, the method is classified as poor and may compromise the dependability of patient results. A Sigma value below two is regarded as unacceptable, suggesting that immediate corrective measures or method replacement are necessary.

Table 1. Interpretation of analytical performance based on Sigma metrics

Sigma range	Quality level
Sigma ≥ 6	World class
5 ≤ Sigma < 6	Excellent
4 ≤ Sigma < 5	Good
3 ≤ Sigma < 4	Marginal
2 ≤ Sigma < 3	Poor
Sigma ≤ 2	Unacceptable

2.7 Quality Control Strategy and Normalized OPSpecs Charts

The calculated Sigma values were used in conjunction with Normalized OPSpecs charts to determine the optimal QC rules and frequency. These charts graphically represent the probability of error detection and false rejection for various QC rules at different levels of precision and bias. The normalized OPSpecs charts represent the probability of error detection, expressed as the percentage of quality assurance (%QA), whereby higher %QA values denote an increased likelihood that quality control rules will detect analytical errors

3 RESULTS

3.1 Determination of Analytical Performance of HbA1c

At QC Level 1, Sigma score displayed marked variability across the study period, ranging from 3.30 in February to 9.01 in August. In February (3.30), April (3.62), and May (3.49), performance results fell within the “marginal” quality range, indicating reduced reliability and increased

susceptibility to both systematic and random error. These months of lower performance were attributable to elevated bias (2.52–3.11%) combined with high imprecision reflected by CV% values reaching up to 1.74. In contrast, results for March (4.59), June (4.60), and July (4.44) indicate "good" performance, while August reached "world class" quality with a Sigma score of 9.01, supported by an exceptionally low CV% of 0.59%. (Table I, Figure I). The fluctuations of Sigma score highlight the method's sensitivity to minor analytical variations at lower analyte concentrations, emphasizing the need for more vigilance monitoring during periods of low Sigma performance.

Table 2. TEa%, Peer Group Mean, Laboratory Mean, Bias%, CV% and Sigma value for Level 1 QC

Month	TEa %	Level 1				
		PGM	LM	Bias %	CV %	Sigma
Feb	8	5.15	5.02	2.52	1.66	3.30
March	8	5.14	5.05	1.75	1.36	4.59
April	8	5.14	4.98	3.11	1.35	3.62
May	8	5.17	5.07	1.93	1.74	3.49
June	8	5.17	5.07	1.93	1.32	4.60
July	8	5.18	5.03	2.90	1.15	4.44
August	8	5.23	5.09	2.68	0.59	9.01

Abbreviations: PGM, Peer group mean; LM, Laboratory mean

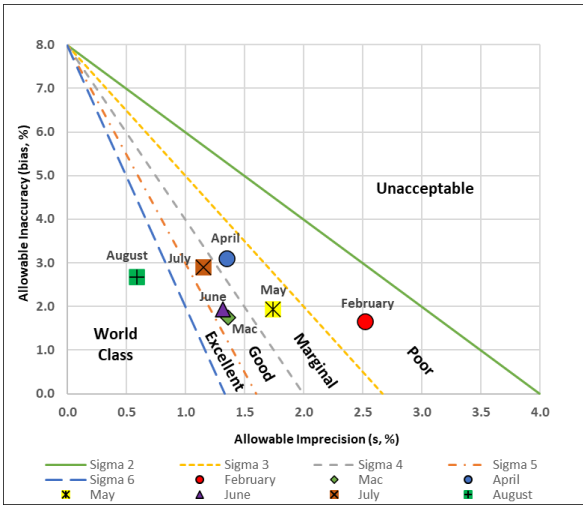

Figure 1. Sigma performance chart for HbA1c Level 1 QC

In contrast, QC Level 2 demonstrated far more consistent and robust performance results. Across all months, Sigma scores ranged from 5.63 to 14.70 (Table II, Figure II). The lowest value, observed in April (5.63), still within the "excellent" category, while March (14.58) and July (14.70) represent peak performance, exceeding the "world class" threshold by a wide margin. Bias remained consistently low (0.20–0.79%), and imprecision was minimal (CV% 0.50–0.93), confirming that the assay achieved both high accuracy and precision at higher analyte concentrations. This stability across months suggests that the method is more robust at Level 2, and less susceptible to minor fluctuations in analytical conditions.

Table 3. TEa%, Peer Group Mean, Laboratory Mean, Bias%, CV% and Sigma value for Level 2 QC

Month	TEa %	Level 1				
		PGM	LM	Bias %	CV %	Sigma
Feb	8	10.06	10.12	0.60	0.93	7.99
March	8	10.07	10.09	0.20	0.54	14.58
April	8	10.08	10.00	0.79	1.28	5.63
May	8	10.12	10.05	0.69	0.96	7.65
June	8	10.12	10.05	0.69	0.60	12.10
July	8	10.07	10.00	0.70	0.50	14.70
August	8	10.05	10.10	0.50	0.63	11.98

Abbreviations: PGM, Peer group mean; LM, Laboratory mean

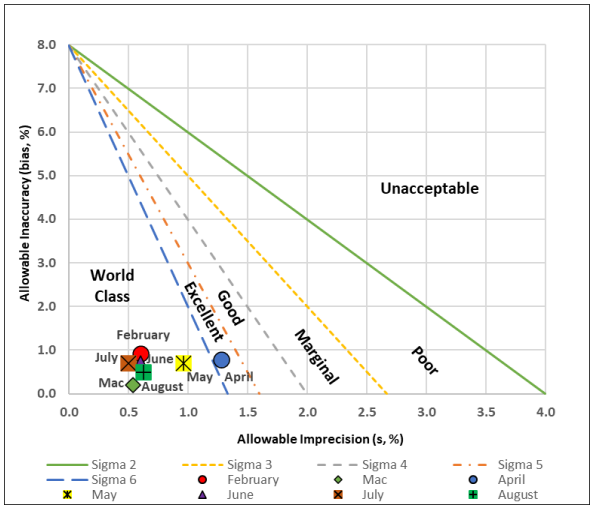

Figure 2. Sigma performance chart for HbA1c Level 2 QC

When the performance of the two QC levels was assessed in parallel, a concentration-dependent trend became evident (Figure III). For QC Level 1, the average Sigma score was 4.27, corresponding to the "good" performance

category. Whereas, for QC Level 2, the average Sigma was 9.56, placing performance in the "world class" range (Table IV). Similarly, the average bias was higher at Level 1 (2.40%) compared to Level 2 (0.59%), while the average CV% was also greater at Level 1 (1.31%) than at Level 2 (0.77%). Collectively, these findings indicate that the HbA1c method performs with greater precision and accuracy at higher concentrations, while performance at lower concentrations is more variable and more susceptible to error.

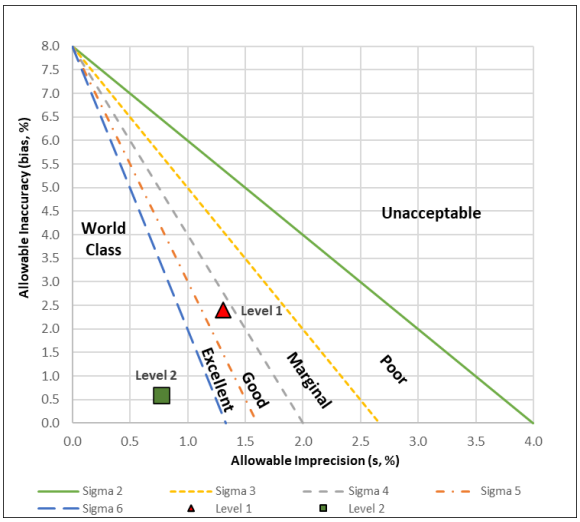

Figure 3. Average Sigma performance chart for HbA1c for both Level QC

Table 4. Summary of QC Performance Metrics and Calculated Sigma Values

Parameter	Level 1	Level 2
Average Bias%	2.40	0.59
Average CV%	1.31	0.77
Average Sigma	4.27	9.56

3.2 Determination of Optimal QC Strategy

The optimal QC strategy for HbA1c assay monitoring was determined using normalized OPSpec chart (Figures IV and V). For Level 1, the chart indicated that applying the $1_{3.5s}$ rule with n = 2 is the most suitable QC strategy, ensuring error detection greater than 90% while minimizing the risk of false rejections. Whereas for Level 2, the OPSpec chart supported the use of the 1_{2s} rule with n=2, which remains sufficient to maintain error detection above 90% while reducing the complexity and frequency of QC monitoring.

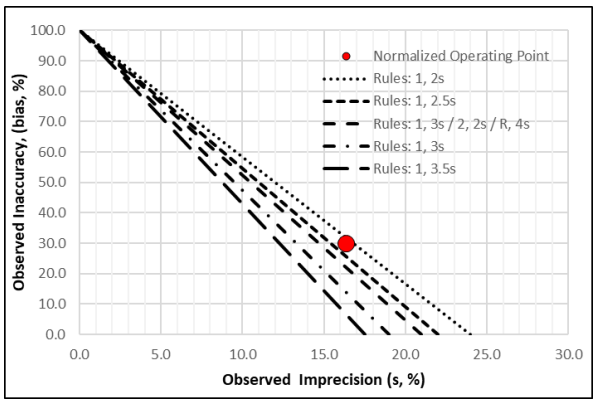

Figure 4. Normalized OPSpec chart (n=2, 90% QA) for Level 1 QC

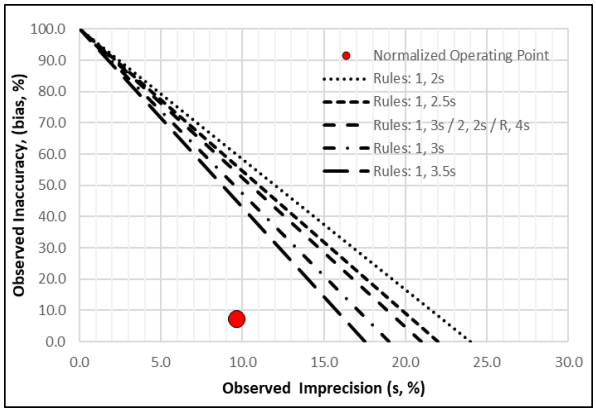

Figure 5. Normalized OPSpec chart (n=2, 90% QA) for Level 2 QC

The recommended QC strategies derived from Sigma performance are summarized in Table V. At Level 1, where the performance is moderate and variability is higher, stricter QC oversight is required through the 1_{3s} rule. In contrast, Level 2, characterized by consistently high performance, allows more flexible monitoring with the 1_{2s} rule. Taking together, these results provide a comprehensive overview of the HbA1c assay performance characteristics, demonstrating that while the system can achieve world-class quality under optimal conditions, its stability is strongly influenced by analyte concentration.

Table 5. QC rules and monitoring frequency based on Sigma metrics

QC Level	Sigma Level	Recommended Control Rule	Detection Level
Level 1	4.27	Apply $1_{3.5s}$ rule, n = 2	QA Level ≥ 90%
Level 2	9.56	Apply 1_{2s} rule, n = 2	QA Level ≥ 90%

4 DISCUSSION

The evaluation of the HbA1c assay using Sigma metrics provides valuable insight into its analytical performance across different concentration levels. The findings clearly demonstrate a concentration-dependent pattern, with QC Level 2 achieving consistently superior performance compared to QC Level 1. In addition, the average Sigma scores for Level 1 achieved 4.27 ("good"), while QC Level 2 achieved 9.56 ("world class"). These results are consistent with those of who reported Sigma-metric score for different concentration points and confirmed that lower concentrations tend to yield weaker performance despite overall strong results at higher analyte levels (13). Collectively, these findings indicate that HbA1c assay perform reliably at higher analyte concentrations than lower concentrations, underscoring the importance of considering analyte concentration when assessing method reliability.

The variability observed at QC Level 1 is particularly important from a clinical standpoint. HbA1c is widely used both for the diagnosis of diabetes and for long-term glycemic control monitoring, meaning that results near the clinical decision thresholds are especially critical. As the range of QC Level 1 is often near the clinical decision cutoff (e.g., 6.5% for diabetes diagnosis, 5.7% for prediabetes), the reduced Sigma performance at low concentrations, coupled with higher bias (up to ~3.11%) and imprecision (CV% up to ~1.74), increases the risk of generating results that could misclassify patients. For instance, patients whose true values are near diagnostic or therapeutic cutoffs may be wrongly labeled as non-diabetic or diabetic, or as well-controlled vs poorly controlled. Such misclassification can influence treatment decisions, potentially leading to overtreatment or undertreatment.

Cas *et al.* (2023) reported that analytical performance (bias and imprecision) of the method may increase the risk of HbA1c misinterpretation, whereby bias has a more significant impact than imprecision when using HbA1c for diagnosis of diabetes (14). As HbA1c near diagnostic cutoff is more likely to classify a patient erroneously, it is essential to use additional modalities such as OGTT for confirmation of diabetes (4), especially when the method displays low analytical performance at lower concentrations.

A study conducted in Malaysia reported that 8.56% of HbA1c samples processed at Hospital Pakar Universiti Sains Malaysia (HPUSM) showed analytical interference, with 0.46% of results being non-reportable (15). This indicates that even with reliable platforms such as the Biorad D-10 HPLC, low-level results can be particularly vulnerable to interference, underscoring the need for stricter QC measures especially for low Level QC. More importantly, analytical interference often produces spuriously low HbA1c results, particularly in patients with chronic diseases associated with reduced red blood cell lifespan. In such cases, unstable assay performance at low concentrations can further compromise the ability to detect the interferences, thereby increasing the risk of result misinterpretation and potential misdiagnosis.

In contrast, the strong and stable performance at QC Level 2 illustrates the robustness of the assay at higher HbA1c concentrations. With Sigma scores consistently exceeding 10, the system demonstrated world-class performance, supported by very low bias (≈0.20%–0.79%) and minimal imprecision (CV%< 1%). This consistency indicates that fewer QC interventions are needed at high concentrations, and the use of relaxed QC rules (e.g. the 1_{2s} rule) is reasonable without compromising patient safety. Doventas and Erdogan (2022) similarly noted that HbA1c analyzers evaluated with Sigma metrics generally perform well when bias and imprecision are minimized, but that the choice of TEa and the concentration under test strongly influence classification (16). Locally, Nor *et al.* (2021) showed that even in challenging biochemical conditions such as high serum urea, HbA1c results obtained by HPLC and capillary electrophoresis were comparable and suggesting that methods can remain robust under specific stressors if proper analytical controls are maintained (11).

The implications of these findings extend beyond QC planning. They provide a framework for laboratories to design concentration-specific QC strategies that balance efficiency with patient safety. By tailoring QC rules to Sigma performance, laboratories can minimize unnecessary costs and workload while maintaining high standards of results reliability. Additionally, the findings also emphasize the need for continuous performance monitoring. Even assays capable of world-class quality may fluctuate over time, and if left unchecked, may lead to compromised patient care. This is particularly relevant in Malaysia, where substantial proportion of patients with T2DM have suboptimal glycemic control. For instance, Ismail

et al. (2011) reported a median HbA1c of 7.4% among patients attending an urban health clinic in Kuala Lumpur, while another study in Johor public clinics found a mean HbA1c of 7.8% of patients above the diagnostic threshold of 6.5% (17). Such findings emphasize how even modest analytical errors can significantly affect patient diagnosis and management.

Integration of Sigma metrics into laboratory practice has been demonstrated as a valuable approach as it offers a structured and quantitative framework for assessing analytical quality and guiding improvement strategies (18). Nevertheless, caution is warranted against relying solely on this approach, as Sigma metrics may oversimplify the complexities of laboratory processes (9). Sigma scores are highly dependent on TEa selection, bias estimation, and concentration-related imprecision, which limits the ability of a single score to fully represent assay quality. Thus, Sigma metrics should be viewed as a complementary tool, used alongside other quality indicators within a comprehensive quality management framework.

The integration of Six Sigma methodology into laboratory practice has been demonstrated as a valuable strategy for ensuring analytical reliability and continuous quality improvement. Originating from industrial process control, the Sigma metric provides a structured and quantitative framework that integrates bias, imprecision, and total allowable error to objectively assess analytical performance. Numerous studies have highlighted their role in enhancing quality management systems and identifying areas requiring improvement (9, 19). Beyond clinical chemistry, the applicability of Sigma metrics has also been extended to other laboratory disciplines, including clinical microbiology, where it has proven useful for evaluating analytical performance and supporting continuous process optimization (20).

Although our study findings provide valuable insight, several limitations remain to be addressed. First, the evaluation utilized QC materials rather than patient samples. While QC data provides an objective measure of analytical performance, real-world clinical variability, including haemoglobin variants and metabolic interferences, may introduce additional challenges that may not be captured during the study period. Secondly, the study was restricted to one analyzer platform, which may limit the generalizability of the results to other HbA1c methods. Future research should address these limitations by incorporating patient-based quality control, comparing performance across multiple analyzers or assay types, and exploring the impact of different TEa standards (e.g. CLIA vs biological variation) on Sigma scores and QC strategies (14, 19).

5 CONCLUSION

This study demonstrates that the HbA1c assay on D-10 HbA1c analyzer delivers consistently superior performance at higher analyte concentrations, with Sigma scores predominantly in the excellent to world-class range. While performance at lower concentrations is less consistent, spanning from marginal to world-class. These findings emphasize the importance of continuous quality monitoring and targeted corrective measures at lower levels, where analytical vulnerability is at the greatest point. Overall, we strongly recommend incorporating Sigma metrics as part of a broader quality management strategy, complemented by additional performance indicators, to ensure reliable clinical decision-making and to safeguard patient safety. The use of Sigma metric grading system not only enables laboratories to determine whether their methods meet the standards required for dependable patient care but also provides practical guidance in designing appropriate QC strategies, as the frequency and rigor of QC depend on the Sigma level achieved. In this way, applying Sigma metrics offers a structured and objective evaluation of analytical performance of HbA1c assay and supports continual improvements in the laboratory quality.

ACKNOWLEDGEMENT

The authors wish to acknowledge the staff of the Integrated Unit (Chemical Pathology, Haematology, and Immunology), Clinical Diagnostic Laboratory (CDL), USM Medical Centre Bertam (PPUSMB), for their valuable technical assistance and support during the evaluation. Their contributions were instrumental to the successful completion of this validation study.

CONFLICT OF INTEREST

No conflict of interest to declared.

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