Nigerian Medical Journal
NIGERIA MEDICAL ASSOCIATION
Print ISSN 0300-1652, E-ISSN 2229-774X

Original Research

Verification of the HbA1c method on the STANDARD F2400® analyzer in a Nigerian Laboratory

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Abstract

Background: For methods that report quantitatively, an assessment of their imprecision and bias should be assessed in the laboratory before their deployment into routine service. This study assessed these parameters of the HbA1C method on the STANDARD F2400® point of care analyzer. These parameters were further combined to generate sigma metrics for the method.

Methodology: An external quality assurance (EQA) material from the Randox International Quality Assessment Scheme (RIQAS) was analysed according to the EP15 protocol of the Clinical Laboratory Standards Institute in SYNLAB Nigeria Laboratory Quality Assurance Department. Estimates of precision and an assessment of bias were determined from the data which consisted of 5 replicates per day for 5 consecutive days. Precision estimates were compared with manufacturer-provided information and estimates of bias were compared with the verification interval for the target value provided by RIQAS. Sigma metrics were determined for total allowable error (TAE) of 8% and 10%.

Results: The grand mean (standard deviation) for the study was 4.95 (0.15) %. The Within-run CV and within laboratory CV were 1.28% and 1.86%, respectively. These were within the manufacturer claims of 1.70% and 1.90%, also respectively. The target value by RIQAS was 5.04(0.24) % with a calculated verification interval of 4.95 - 5.13%. The sigma metrics for the method at TAE of 8% and 10% were 4.8/3.3 and 6.3/4.3 within the run/within laboratory estimates respectively.

Conclusion: The HbA1C method on the STANDARD F2400® Analyzer displayed performance characteristics that are consistent with manufacturer specifications and are above industry standard quality for a point-of-care device for HbA1C. These suggest that may be used to support routine monitoring of persons with diabetes mellitus in Nigeria.

Keywords: Imprecision; Bias; Sigma Metric; Method Verification; HbA1C; POCT.

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How to cite: Kuti MA, Adeleye JO. Verification of the HbA1c method on the STANDARD F2400® Analyzer in a Nigerian Laboratory. Niger Med J 2025; 66 (1):246-255.https://doi.org/10.71480/nmj.v66i1.698.

Quick Response Code:

Introduction

The international standard for quality and competence in medical laboratories, ISO 15189, stipulates that medical laboratories should use examination methods that have been validated for their intended use to assure the clinical accuracy of the examination for patient testing.[1] The product of the validation experiments as conducted by the manufacturer are performance specifications claims with regards to measurement trueness, measurement precision including measurement repeatability, and measurement intermediate precision, analytical specificity, including interfering substances, detection limit, quantitation limit, and any other parameter that are relevant to the particular method.[2] The ISO standard further requires that before these validated examination methods are used by the laboratory for reporting on patient samples, the laboratory must verify that the performance specifications reported by the manufacturer are obtainable in the laboratory. [1] Verification experiments are conducted by the laboratory and the results are reviewed to check if the levels obtained are within the variance stated by the manufacturer and therefore meet the requirements for the intended use. [3] While the scope of the verification experiments is usually narrower than validation experiments, they should be focused on performance claims that are relevant to the intended use of the examination results. [4]

For methods that report quantitative information, measurement precision and measurement trueness are particularly important. Precision is the closeness of agreement between indications or measured quantity values obtained by replicating measurements on the same or similar objects under specified conditions. [5] It is an indication of the stability in the measuring system that minimises randomness in the result of the laboratory. Its quantitative expression, imprecision, may be reported as standard deviation or coefficient of variation of the replicate measurements. In the laboratory, Imprecision has several components which include repeatability, intermediate precision, and reproducibility, depending on the period over which the measurements are made and variations in the measuring system such as environmental conditions, analysts, laboratories, and reagent lots.[6] Repeatability is assessed within a single run while intermediate precision combines within a run and between run precision and reflects within laboratory CV. Reproducibility is variation when the analytical conditions vary in the number of analysts, environmental conditions, lot of reagents, different equipment, and different laboratories. An assessment of imprecision is particularly important when repeated measurements of a test are used to monitor the effectiveness of an intervention or clinical course of a disease over a period. [7] Measurement trueness is defined as the closeness of agreement between the average of an infinite number of replicates measured quantity values and a reference quantity value. It is quantified as measurement bias which is a measure of systematic error. [6] An appreciation of bias is important to ensure that a measuring system is not systematically under- or over-reporting to a clinically significant degree. The degree of imprecision and/or bias associated with a laboratory's method are therefore key characteristics with potential for immediate impact on clinical decision making.

The clinical laboratory standard institute has published a procedure for the verification of precision and estimation of bias of clinical methods which combines the 2 activities into one single experiment. [8] Among several approaches to conducting this experiment is the use of proficiency testing/external quality assurance (PT/EQA) materials. PT/EQA programs often use a target value that is the mean of results of a large identifiable number of laboratories with a given measurement procedure or procedures that may be considered equivalent. Reliable estimates for the standard errors of these target values (TVs) may then be obtained from reported standard deviations (SD) and the relevant numbers of participating laboratories. The objectives of the study were to determine the precision and bias estimates of the HbA1c method of the Standard F2400 analyser in a Nigerian Laboratory and to use these parameters to determine the sigma metrics of this point of care device.

Methodology

Ethics: Ethical approval was not requested for this study. This was an equipment verification study that did not involve the use of any human or animal subjects or data derived from them. The material used for the study was historic lyophilised external quality assurance material.

The verification experiment was carried out in the quality assurance department of Synlab Nigeria Laboratories in Ilupeju. One staff member trained and deemed competent by the local vendors of the SF2400 was responsible for all the analysis. All the analysis were performed in a climate controlled room with temperatures maintained between 22 and 24 degrees Celsius.

The equipment and its reagents

STANDARD F2400 Analyzer is a fluorescence immunoassay device that can perform qualitative and quantitative analyses for infections, respiratory diseases and chronic diseases. It is manufactured by SD Biosensor, Republic of South Korea. It is currently listed among the methods certified by the NGSP. [9] The serial number of the equipment used for the verification experiment was FA24C02AA0269. It was provided by Codix Pharma Limited who are the authorised representatives of SD Biosensor in Nigeria. The company also provided the STANDARD™ F HbA1c reagent packs used for the analysis. The lot number of the kit used for the analysis was 6074431AC with a stated expiry date of 10/02/2024.

The external quality material used for the study was sourced from the Randox International Quality Assessment Scheme (RIQAS). The sample was that of sample 8, cycle 18. The material was whole blood, and it was used as provided. The comparative instrument was the Roche C4000 as recommended by the manufacturer, SD Biosensor.

Study design

Precision and Bias Studies

The CLSI EP-15 protocol allows for the simultaneous verification of a manufacturer's claims for the precision of a measurement procedure and the trueness of the measurement procedure relative to the assigned values of materials with known concentrations. It requires 25 or more measurements of a material with known concentration and uncertainties, by the candidate procedure, made over five or more days. Using the data obtained, within and between laboratory precision estimates are calculated and compared with the manufacturer's claim in its information for users (IFU) leaflet. In this experiment where EQA materials were used, bias estimates were determined by comparing the grand mean of the experiment considering the uncertainties involved in all the measurements performed, against the mean of reported results for the Roche C4000 for sample 8, cycle 18 of the Glycated Haemoglobin program of RIQAS.

Sigma Metric

The Medical decision chart of Westgard was for determining the Sigma metric. The Excel sheet was downloaded from https://westgard.com/downloads.html. Total allowable errors of 8% and 10% for HbA1C [10] were used for the calculations.

Statistical analysis.

The presence of potential outliers in the replicates was assessed by Grubb's test. The outlier limits were determined from the calculated mean and SD using the Grubbs' factor at a 99% confidence interval. One-way analysis of variance was used to determine within-run and between-run estimates of imprecision. The CLSI protocol for estimation of bias involves the use of the target value, which is the assigned value of the EQA material by Randox EQA, and the calculated mean of all the replicate values. The difference (bias) between the two values is calculated. This was followed by a calculation of the standard error of

this difference and then a verification interval (VI) that has a 95% probability of containing the true difference. The GM is then assessed considering the verification interval and allowable error limit. If the GM falls within the verification interval, it is deemed acceptable. An acceptable bias percentage was calculated as below:

$$\text{Acceptable percentage bias} = \frac{\text{Target Value} - \text{Lower Verification Limit}}{\text{Target Value}} \times 100$$

$$\text{Actual percentage bias} = \frac{\text{Target Value} - \text{Grand Mean}}{\text{Target Value}} \times 100$$

Results

Table 1 shows the results of the 25 data points. The mean (standard deviation) of all the values was 4.95 (0.15) %. The target value (standard deviation) of the EQA material was 5.04(0.24) %. This was generated from the submitted results of 2,088 laboratories. Grubb's factor (G) for 25 values is 3.135 (99% CI). Grubb's limits were calculated as mean $\pm$ G x SD. Grubb's lower and upper limits were 4.48% and 5.42%, respectively. This indicates that there were no outliers within the data set. The bias between the GM and the TV was 1.79%.

Table 1: Study Data

	Run 1	Run 2	Run 3	Run 4	Run 5
Day 1	5.10	5.10	5.10	4.90	4.70
Day 2	5.20	5.00	4.90	4.80	4.70
Day 3	5.10	5.10	5.00	4.80	4.80
Day 4	5.10	5.00	5.10	4.80	4.80
Day 5	5.00	5.10	5.00	4.80	4.80

All values are in %

From the information for the user leaflet in the reagent pack, the manufacturer claimed a within-run CV and a within the laboratory of 1.70% and 1.90% for HbA1C of 5.2%. Table 2 shows the result of a one-way analysis of variance of the data set. Within-run variance (repeatability), between-run variance, and within-laboratory variance (within-run variance plus between-run variance) was calculated as 0.00400, 0.00446, and 0.00846 respectively. Converting to the coefficient of variation gave a within-run CV, between-run CV, and within-laboratory CV of 1.28%, 1.35%, and 1.86%, respectively. Furthermore, to achieve a 95% confidence level, the upper verification limit for within-run CV and within-laboratory CV was calculated. These were 2.13% and 2.39%, respectively.

Table 2: Analysis of Variance Table

Source of Variation	***Sum of Squares***	***Degrees of freedom***	***Mean Squares***
Between Groups	0.4624	4	0.1156
Within Groups	0.08	20	0.004
Total	0.5424	24	

Using the data from the EQA provider: the target value, its standard deviation, and the number of laboratories that submitted results, a verification interval (VI) for the TV. The VI has a 95% probability of containing the true difference between the TV and the GM. The calculated VI was 4.95 - 5.13%.
The acceptable percentage bias was [(5.04 – 4.95)/5.04] X 100 = 1.78%and the actual percentage bias was [(5.04 – 4.95)/5.04] X 100 = 1.78%.

Table 3 is a summary of the results of the entire verification experiment. It indicates that the precision claims of the manufacturer were verified to be attainable in the laboratory and the bias estimates demonstrated were acceptable. Figures 1 and 2 are the method decision charts with TAE set at 8% and 10% respectively. The sigma metrics for the assay at TAE of 8% were 4.8 and 3.3 for the within-run and within-laboratory estimates respectively. For TAE of 10%, the estimates were 6.3- and 4.3-sigma metrics.

Figure 1: Method Decision Chart – Total Allowable Error at 8%

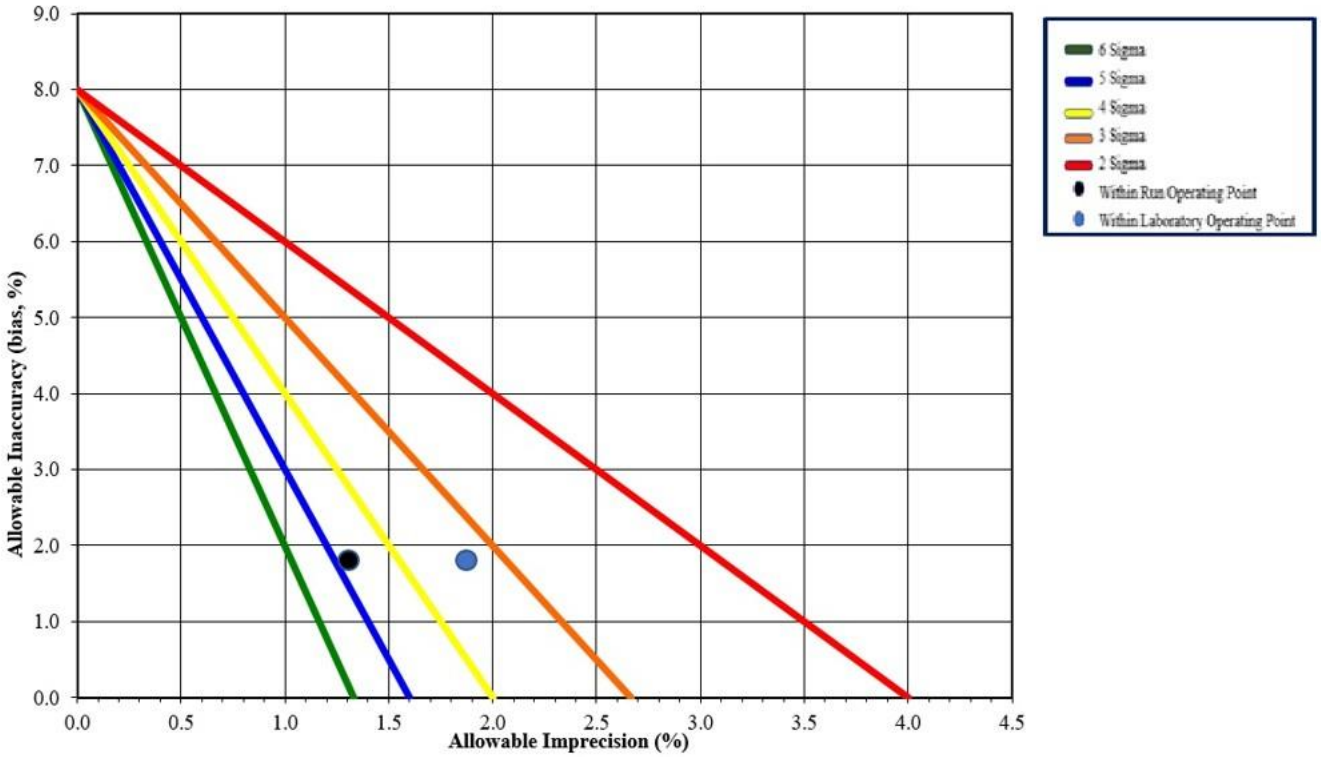

Figure 2 Method Decision Chart – Total Allowable Error at 10%

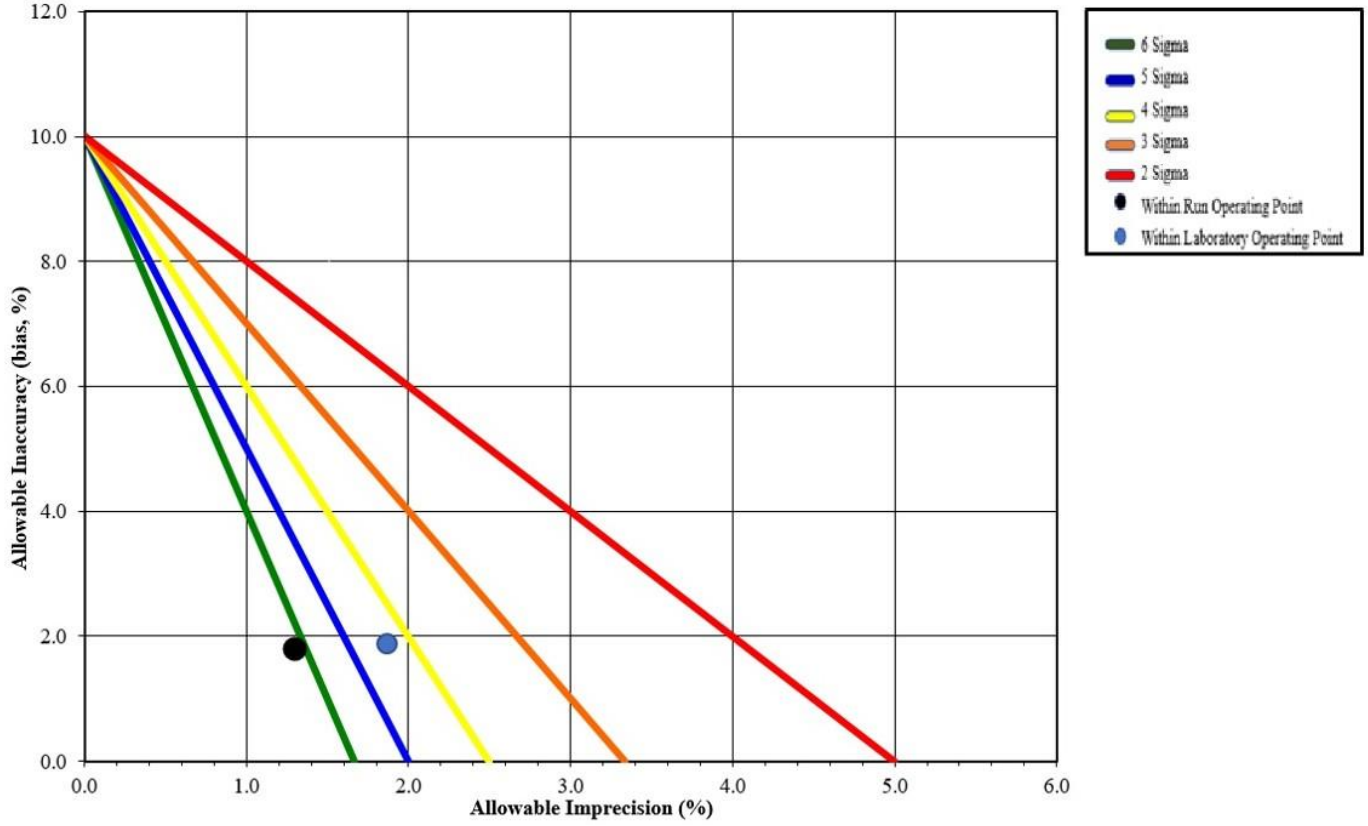

Table 3: Summary of Results

Verification of Precision			
Repeatability	UVL(CV%)	Study (CV%)	Comment
	2.1	**1.3**	Pass
	IFUCV% –1.7Level1–5.2%		
Within Laboratory	UVL(CV%)	Study (CV%)	
	2.4	**1.9**	Pass
	IFUCV% –1.9Level1–5.2%		
Bias Estimate	Verification Interval	Study	
	4.95–5.13	**4.95**	Pass
	Target Value 5.04%		
		Comparison Method: RocheC4000	

UVL – Upper verification limit; CV – Coefficient of variation; IFU – Information for users

Discussion

The within-run and within-laboratory precision estimates obtained in the study were lower than manufacturer estimates. The bias estimates were also within acceptable variation from the comparison instrument. These results from this CLSI EP-15 A3 protocol study demonstrate that the precision claims of the manufacturer for the measurement of HbA1c on the STANDARD F2400 (SF2400) Analyzer are achievable in a Nigerian Laboratory in the hands of a trained Nigerian operator. They further indicate that, in these circumstances, this tabletop device is able to achieve an acceptable level of bias when

compared to a central laboratory instrument like the Roche C4000. The European Federation of Laboratory Medicine (EFLM) publishes analytical performance specifications (APS) based on biological variation. They use formulae by Frader and Petersen [11,12] for imprecision and bias as well as estimates of within-subject and between-subject biological variation estimates derived from a meta-analysis of current and relevant literature. They state that the minimum specifications for HbA1C methods are an analytical imprecision CV of 1.2% and a bias of 2.7%. [13] Although its within-run estimates for this study are slightly higher than this, the bias estimate of the device found in this study is within EFLM's acceptable limits. There have been other studies reporting on the evaluation of the performance of HbA1c platforms in the routine laboratory. Using the CLSI protocol, Chakravarthy et al reported an evaluation of the central laboratory-sized VITROS 5600.[14] It is immunoassay-based methodology had an acceptable performance compared to the manufacturer's claims. Their demonstration within laboratory precision for the analyser was a CV of 0.6% compared to a manufacturer's claim of 1.1%. The actual bias percentage of their study was 0.14% compared with an acceptable percentage bias of 0.36%. They did, however, note, as in the CLSI guidelines, that their use of a peer group mean of a QC material obtained in an interlaboratory QC program was a major limitation. In this study, we used a more reliable estimate from a major external quality assurance provider. Differences in the conclusions obtainable from the 2 types of materials have been demonstrated. [15] This is because the estimates from interlaboratory QC programs may be skewed because the participating laboratories may differ markedly from one another.[14]

The International Federation of Clinical Chemistry (IFCC) Task Force on implementation of HbA1c standardization has proposed the use of sigma metrics as the method of choice for describing quality targets for the analyte. [16] They suggested a minimum of 2-sigma and 4-sigma as the desired quality target for methods to be used in routine laboratories and clinical trials, respectively. These targets represent a failure rate of about 5 in 100 and 6 in 1000, respectively. The sigma metrics demonstrated in this study suggest that the HbA1c on the SF2400 may be suitable not just for routine laboratory use but may also support clinical trials. Lenters-Westra et al have conducted periodic evaluations of the performance of several POCT devices for HbA1C over a 15-year period [17–20]. They note that despite improvements in technology and quality, there remain commercially available platforms with unacceptable performance characteristics. An awareness of this by the clinical and scientific community should guide their use of these POCT platforms for screening, diagnosis, and monitoring for DM. [20] As the sigma metric reflects error rates, it has also been recommended to provide guidance for the internal daily quality control plan that should be implemented to monitor the quality of routine operations. [21] A higher sigma metric indicates a more stable method and less rigorous DQC monitoring. Given the sigma metric demonstrated by the SF 2400 HbA1c in this study, 3.3-sigma for within laboratory estimates, a sufficiently sensitive DQC can be run twice per day with two levels of QC per day and the use of a multi-rule system. For devices with <3-sigma, DQC may need to be run three times per day with three levels with considerations for testing in duplicate as well as the maximum QC rules. [22] This may have significant financial implications for the laboratory.

The selection of laboratory devices to support the management of diabetes mellitus is important. A diagnosis of the disease cannot be made in the absence of biochemical measurements of either glucose or HbA1C. Furthermore, an increasing array of tests are required for defining aetiology, guiding therapeutic decisions as well as detection of complications. [24] Point of care testing, defined as any investigation carried out in a clinical setting or the patient's home for which the result is available without reference to a laboratory and perhaps rapidly enough to affect patient management has been shown to have many benefits in the clinical care of people with diabetes mellitus. [24,25] A major benefit of POCT is the ability to deliver immediate results that accelerate clinical decision-making at the time of the patient visit. [25] The immediacy of the impact of these POCT devices and the consequences of the same should require that these instruments have quality characteristics that approach those instruments used in the main laboratory. Studies that have examined the quality of several central laboratory instruments for

HbA1c have almost uniformly demonstrated sigma metrics exceeding 3-sigma. Wang et al, using aTAE of 7%, showed that 4 out of 6 central laboratory grade instruments (Bio-Rad Variant II automatic, Bio-RadVariant II Turbo 2.0, SEBIA Capillary's 2 Flex Piercing system, Trinity Biotech Ultra2, Trinity Biotech Hb9210, and Roche Modular PPI detection system) demonstrated a sigma metric of at least 3.5. Similarly, Maesa et al, with TAE set at 10%, showed that 3 out of 4 of the equipment examined had a sigma metric of at least 6, using within laboratory imprecision and bias estimates. [26] This would suggest that while the SF2400 may have desirable characteristics for a POCT device, it may still not replace central laboratory analysis. This is consistent with a current opinion that while POCT devices may support glycemia monitoring in persons with diabetes mellitus, there remains concern about their use for the diagnosis of the disease. [27] The *cost of* POC HbA1ctesting, which can be several-fold more expensive than testing in a central laboratory is an important consideration. Although the current study did not include a cost evaluation, there is evidence that with adequate test numbers cost parity with central laboratory may be achieved in poor and remote areas in low-middle income countries that may support their inclusion in public health programs. [27,28]

In conclusion, the SF2400 shows significant promise as a tool in supporting the management of persons with diabetes mellitus. It shows desirable performance characteristics that indicate above-industry standard quality for a POCT device. Current concerns about POCT devices, in general, may limit their use for the diagnosis of diabetes mellitus.

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