

The Use of Internal and External Quality Control Data for the Calculation of Measurement Uncertainty of Glycated Hemoglobin

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Diabetes mellitus is a burgeoning worldwide problem. For monitoring the glycaemic state in diabetes, glycated hemoglobin (HbA1c) is one of the standardized tests. Measurement of uncertainty shows the magnitude of the doubt about a measurement result. The current study aims to estimate the measurement uncertainty (MU) of HbA1c in our laboratory using the method suggested by EURACHEM/CITAC and advocate its utilization as a quality standard in clinical laboratories as part of the quality control program to enhance the reliability of their results. The HbA1c levels were measured using a high-performance liquid chromatography method. The “Top Down” technique proposed by EURACHEM/ CITAC was used to calculate the uncertainty of HbA1c. This method allows the inclusion of the bias and imprecision of the HbA1c method. Bias value was calculated from data of external quality assessment results of the EQAS program and two levels of internal quality control results data was used to calculate the imprecision. The measurement uncertainty (95% confidence interval) of HbA1c was estimated to be 4.2%. When measurement uncertainty was taken into account, the acceptable range for an HbA1c value of 6.5% typically used to diagnose DM was between 6.2 to 6.77. The EURACHEM/CITAC method is valuable for calculating MU of HbA1c and is a viable way that can be utilized as an extra analytical target in HbA1c testing. Furthermore, the data can assist clinicians in determining the degree of confidence that may be placed in test results.

Keywords: EURACHEM/CITAC; Glycated hemoglobin; Measurement uncertainty; Top-down approach.

Diabetes mellitus (DM) is a multifactorial chronic metabolic disorder characterized by persistent hyperglycemia affecting large part of the population¹. According to the International Diabetes Federation (IDF), nearly 463 million people aged 20-79 years had diabetes in 2015, with the figures predicted to increase by another 200 million by 2040 if current trends persist.

The use of glycated haemoglobin for monitoring diabetes started in the 1980s. In 2009, the World Health Organization issued guidelines on the utility of HbA1c as a diagnostic test. The guidelines also stressed upon the need for maintaining stringent quality assurance measures of the measurand². Pre-diabetes is an asymptomatic type of diabetes mellitus in which blood glucose

levels are elevated but not high enough to be classified as diabetes. Pre-diabetes can be clinically identified through HbA1c values between 5.7% and 6.4% (3). Type 2 Diabetes mellitus (T2DM) is diagnosed when the HbA1c level exceeds the pre-diabetes values (e.g. 6.5%)⁴.

In recent years, since the HbA1c values produced by clinical laboratories have such a significant impact on the diagnosis of diabetes and patient monitoring, it is critical for any clinical laboratory to continuously monitor the performance of their methods, ensuring that these methods achieve proper analytical performance and ensure that the results are accurate⁵.

Measurement uncertainty is defined as the “nonnegative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used” according to the Guidelines to the Expression of Uncertainty in Measurement” (GUM) which was first published in 1993^{6,7}. Measurement uncertainty indicates with a certain probability that the true value lies within the limits of uncertainty⁸.

The calculation of uncertainty of measurement of glycated hemoglobin by using the method proposed by EURACHEM/CITAC is the core objective of our study and suggesting the adaptation of this parameter as an analytical tool used by clinical laboratories as part of their quality control to ensure the accuracy of their HbA1c results. The knowledge of the interval within which the true value of the glycemic status of the patient lies would enable the clinicians to determine the protocol for treatment with confidence.

MATERIALS AND METHODS

The study was conducted at SRM General Hospital central lab and the data generated from the results of Internal Quality Control at SRM General Hospital Central lab (Biorad laboratory Inc., USA) in the time period from January to December 2020 and External Quality control results provided by Christian Medical College (CMC), Vellore, Tamil Nadu, India.

High performance liquid chromatography method

The high-performance liquid chromatography method (D-10 Biorad laboratories Inc., USA) was used to estimate the HbA1c values.

Estimation of measurement uncertainty

The statistical method used to obtain the uncertainty of measurement of HbA1c was the “top down” approach, described in the EURACHEM/CIATAC guide⁹. This method will allow to calculate the uncertainty of HbA1c by using results of quality control data. Formula for that

$$u_c = \sqrt{u(rW)^2 + u(Bias)^2/2}$$

where the uncertainty component related to random error $u_{(RW)}$ and systemic errors $u_{(Bias)}$ are calculated separately by using the quality control data.

Step-1

The calculation of uncertainty of within-laboratory reproducibility $u_{(RW)}$ used Bio-Rad control level 1 CV% and level 2 CV %. It yields the uncertainty associated with random error.

Step-2

In order to calculate the $u_{(Bias)}$ (which is associated with systemic error), we have calculated RMS bias and RMS u_{ref} which we can calculate by using the data from EQAS.

$$RMS_{bias} = \sqrt{\Sigma bias(eqas)^2/N}$$

(N- number of external quality control, EQAS-external quality control results)

Bias is calculated by using following formula

$$Bias = \frac{Lab\ mean - Peer\ group\ mean}{Peer\ group\ mean} \times 100$$

Step-3

RMS u_{ref} is standard uncertainty component for the certified or assigned value. Calculated by, formula

$$RMS\ u_{ref} = 1.25 \times CV\ \% / \sqrt{N}$$

Step-4

U bias calculated by using the RMS bias and RMS u_{ref} values with the help of the following formula

$$U\ bias = \sqrt{RMS(Bias)^2 + RMS(cref)^2}$$

Step-5

$$Uncertainty\ U = \sqrt{UrW^2 + U\ bias^2/2}$$

For calculating expanded uncertainty include a coverage factor of $K=2$, which provides an expanded uncertainty at approximately the 95% confidence level. Formula is:

$$U = K \times u_c$$

The U results were compared with TAE for the HbA1c test.

RESULTS

The retrospective review study was conducted in the central lab of SRM Medical College Hospital & Research Centre (SRM MCH & RC). The data consisted of 142 level 1 and level 2 internal quality control results and 12 months external quality control results (Table 1 & 2).

The results of the uncertainty estimation of HbA1c in our study are shown in table 3. HbA1c 6.5% is recommended as a cut-off value for

diagnosing DM. The MU for HbA1c was estimated at $\pm 4.2\%$, when the MU was taken into account, the acceptable range for a value of 6.5% was about 6.23 to 6.77.

DISCUSSION

Measurement of HbA1c plays an important role in monitoring the glycemic status of diabetic patients. International organizations like the NGSP and IFCC have issued specific guidelines for standardization of the parameter¹⁰. Individual laboratories should take responsibility to ensure the adherence to the analytical performance goals to achieve HbA1c results that are fit for interpretation purposes.

Various fields of metrology find uncertainty of measurement to be an important parameter. The usage of MU in clinical laboratories

Table 1. Imprecision results of control sera level 1 and level 2

	Control 1	Control 2
HbA1c expected provider concentration	5.4	9.6
Number of IQC samples analyzed	142	142
Mean of our IQC results	5.34	10.01
CV % of our lab	2.22	2.06

Table 2. External quality control assessment program data of 1 year

	Results
Peer group Monthly average participants	483
Peer group coefficient of variance (CV%)	2.45
Peer group HbA1c mean concentration	6.32
HbA1c mean concentration of laboratory	6.40

Table 3. The measurement of uncertainty for HbA1c

Internal quality control	Level 1 (mean and CV%)	5.32 - 2.22%
	Level 2 (mean and CV%)	10.01 - 2.05%
	uRW	2.14
External quality control	EQAS (mean and CV%)	6.32 - 2.45%
	n	483
	RMS bias	2.0
	U_{ref}	0.29
Standard, combined and expanded uncertainty values	Standard uncertainty	2.0
	Combined uncertainty	2.1
	Expanded uncertainty	4.2

is limited. ISO/IEC17025 suggest the inclusion of uncertainty of measurement in test reports whenever relevant¹¹. The complexity of measuring this parameter as given by GUM deterred its usage in many laboratories. The EURACHEM/CITAC guide provides a relatively easy method for calculating uncertainty measurement using the internal & external quality control data.

Within laboratory reproducibility and bias due to the method and laboratory condition can be assessed by the internal & external quality controls. Day-to-day variation and sample repeatability together form the within-laboratory reproducibility. Bias represents the systemic error and bias variation can be evaluated using external quality assessment program¹². We have utilized imprecision, bias, uncertainty of bias in the calculation of MU in the current study to accord the actual dispersion of values pertaining to the measured parameter i.e., HbA1c.

Lenter-Westra et al demonstrated that HbA1c results generated by methods accredited by NGSP are not always useful for clinical usage. Standardization of the methods used for estimation of HbA1c is still insufficient¹³. Assessment of reliability of a test parameter is essential to achieve a level of confidence in the analytical precision of the test result. MU provides the reliability and defines the predicted variability in a laboratory result in case of repetition. Knowledge of MU helps in improved evaluation of clinical decisions.

In 2009, International Expert Committee published a report recommending that HbA1c can be used to diagnose diabetes when the levels are 6.5 %^{14, 15}. Hence, the knowledge of MU becomes important for decisions involving diabetes diagnosis. Unal et al demonstrated the effect of measurement uncertainty on the clinical decision levels of HbA1c in 1555 patients out of the 10212 subjects in a retrospective study¹⁶. In our study, we found the expanded uncertainty to be 4.2%. The uncertainty of HbA1c value was below the total allowable error of $\pm 6\%$ in our laboratory. When it is applied to the HbA1c of 6.5%, the accepted value would be between 6.23 % and 6.77 %. The interpretation of the result should be made with the knowledge of the measurement uncertainty. Assessment of the reliability of a test enhances its utility in the clinical field. There will be alteration in values that are close to the decision limits when

appraised with the measurement uncertainty and requires careful interpretation. Evaluation of HbA1c reports with MU will indicate the actual limits and enable the clinicians to analyse and interpret the reports with a stated level of confidence. A limitation of our study is that the estimation of bias is ideally performed using reference materials which is not viable in most clinical laboratories. Also, matrix related bias was not eliminated since the commutability of control samples was not studied.

CONCLUSION

Our laboratory results demonstrated that the expanded uncertainty is 4.2% which is below the total allowable error of $\pm 6\%$ suggested by NGSP. According to ISO17025, the laboratories should provide MU on request. Periodic calculation of the uncertainty of measurement of glycated hemoglobin is recommended to be a part of quality control program of the laboratory. Awareness of concept of MU would provide the clinician with a level of confidence that can be assigned to the test results and interpret them accordingly.

Conflict of Interest

There is no conflict of interest.

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