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Technical Report

Estimation of measurement uncertainty of glycated hemoglobin at Atellica Solutions

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Abstract

Objectives: Glycated hemoglobin (HbA1c) is a commonly used laboratory test for screening, monitoring, and diagnosing diabetes mellitus (DM). Uncertainty is a statistical expression that shows the effects of factors arising during measurement processes and affecting the reported results. It is important to give reliable laboratory results. This study aims to estimate the measurement uncertainty (MU) of HbA1c at Siemens Atellica Solutions.

Methods: Internal and external quality control results of 6 months data were used to calculate the MU according to the Clinical & Laboratory Standards Institute (CLSI) document EP29-A. The coefficient of variation (CV%) of the two levels of internal quality control materials was calculated. The external quality control results of 6 months data obtained from the EQAS program of Bio-Rad were used to calculate the bias.

Results: The precision results of the assay were within the current National Glycohemoglobin Standardization Program requirement of ≤4%. The HbA1c level above 6.5% is one of the diagnostic criteria of DM. We estimated the expanded uncertainty as 6.40% for HbA1c at the level of 6.5%. It is presented as 6.5±0.4%.

Conclusion: It is important that laboratorians report accurate and reliable test results as clinicians make their decisions based on laboratory data. Laboratories should add measurement uncertainty results as a part of quality control programs. The laboratory tests with clinical decision limits should be presented with measurement uncertainty results.

Keywords: Atellica Solutions, glycated hemoglobin, measurement uncertainty

Diabetes mellitus (DM) is a chronic and degenerative disease accompanied by microvascular and macrovascular complications [1]. All over the world, there are millions of people living with diabetes [1, 2]. The prevalence of DM increases every year, DM accounts for more than 1.5 million deaths every year [1].

Glycemic control is the most important component for reducing the complications of diabetes [1]. The relationship between low glycated hemoglobin (HbA1c) and good glycemic control was shown by the United Kingdom Prospective Diabetes Study and the Diabetes Control and Complications Trial (DCCT) [1, 3, 4]. HbA1c is an important test for screening, monitoring, and diagnosing DM [2]. HbA1c result greater than 6.5% is one of the diagnostic criteria of DM [5]. According to the American Diabetes Association (ADA), HbA1c levels are classified into three groups: value less than 5.7% represents a low risk for DM, 5.7-6.4% shows a high-risk for DM, also called prediabetes, and the cut-off value of HbA1c for diagnosis of DM is ≥6.5% [6, 7].

The clinical laboratories use a lot of analytical methods for measuring HbA1c based on ion-exchange or affinity chromatography, electrophoresis, and immunological principles [8]. Laboratory results have variations that cause uncertainty which must be estimated and regularly reviewed by laboratories [9]. Producing qualified, accurate, and reproducible results is one of the most important tasks of medical laboratories. Uncertainty is a statistical expression that shows the effects of factors arising during measurement processes and affecting the reported results. According to the CLSI EP-29-A guideline, uncertainty defines an interval within which the true value of the measurement is expected to lie with a stated level of confidence [10].

There are three types of uncertainty: standard, combined, and expanded [11]. Standard uncertainty means the imprecision

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determined in the laboratory. Determining the measurement uncertainty of the results produced by the laboratory is very important in verifying the performance of the measurement system. Uncertainty gives the laboratorians a better understanding of the performance and limitations of their methods. Thus, it enables the identification of technical steps in which measurement uncertainty can potentially be reduced. For the safety of the patients, it is critical that most laboratory tests are within the analytical target of their clinical use, as it can significantly affect the clinician's ability to correctly evaluate the patients [10].

In this study, we aimed to demonstrate the calculation of measurement uncertainty of glycated hemoglobin at Atellica Solutions instrument (Siemens Healthineers, Germany).

Materials and Methods

The study was conducted at Ankara City Hospital Central Biochemistry Laboratory. The study was approved by the local ethics committee. HbA1c levels were performed on the Atellica Solutions autoanalyzer (Siemens Healthineers, Germany) via the principle of latex agglutination inhibition assay.

Internal and external quality control results of 6 months data were used to calculate the MU according to the Clinical&Laboratory Standards Institute (CLSI) document EP29-A [10]. According to this approach, the quality control data of the laboratory are used to calculate the measurement uncertainty (MU), using the following general formula:

$u_c=\sqrt{(u_{Rw}^2+u_{bias}^2)}$,

$U=k\times u_c$,

where k is the coverage factor (for 95% level of confidence [CI], k=2), uRw is the relative standard uncertainty due to within laboratory imprecision, which is associated with possible random errors and obtained by the calculation of the coefficient of variation (CV%) of two levels of internal quality control materials (CV_1% and CV_2%).

$u_{Rw}=\sqrt{[(CV_1^2+CV_2^2)/2]}$.

The external quality control results of 6 months data obtained from the EQAS program of Bio-Rad were used for calculating the uncertainty of the bias (u_{bias}) that points out possible systematic errors. The mean square value of the monthly bias results of the laboratory (RMS_{bias}) and the uncertainty of the reference value of the EQAS program (u_{cref}) were calculated. Then, standard ubias was calculated according to the formula:

$u_{bias}=\sqrt{[(RMS_{bias})^2+(u_{cref})^2]}$,

Here,

$RMS_{bias}=\sqrt{[(\Sigma_{bias}(\text{external quality control})^2/N}$,

where N is the external quality control number.

$u_{cref}=(sR/\sqrt{n})$,

where sR is the mean CV% of the external quality control result and n is the number of peer groups participating in the EQAS program.

The combined standard uncertainty, u_c, was quantified.

$u_c=\sqrt{(u_{Rw}^2+u_{bias}^2)}$.

The expanded uncertainty, U, including a coverage factor of k=2, which provides an expanded uncertainty at approximately 95% confidence level, was calculated to include the largest possible number of variables that can influence the uncertainty of the method:

$U=k\times u_c$,

where k is the coverage factor (for 95% level of confidence [CI], k=2).

HbA1c concentration was expressed as the percentage (%) of glycated hemoglobin in relation to the concentration of the total hemoglobin, as suggested by National Glycohemoglobin Standardization Program (NGSP).

The ADA recommends that HbA1c can be used for the diagnosis of diabetes with a threshold of ≥6.5% [7]. The analytical performance specification of measurement uncertainty established for the present study was within the previously suggested goal of HbA1c as ±0.5% [12].

Results

The imprecision results are provided in Table 1. The precision results of the assay were within the current NGSP requirement of ≤4%. The estimated measurement uncertainty results of our method are presented in Table 2. The expanded uncertainty of our method was 6.57%.

Discussion

As recommended by the DCCT, HbA1c is an important marker of glycemic control in DM [13]. The standardization of its measurement is challenging. The National Glycohemoglobin Stan-

Table 1. Imprecision results of internal quality control sera

	Control 1	Control 2
Expected concentrations (%)	5.34	9.66
Number of results (n)	307	307
Mean concentration of results (%)	5.34	9.35
Laboratory mean CV (%)	3.16	2.79

CV: Coefficient of variation.

Table 2. Measurement uncertainty results

Parameters	Values
u_{Rw}%	2.98
RMS_{bias}%	1.17
u_{cref}%	0.07
Combined uncertainty (u_c)%	3.20
Expanded uncertainty (U) %	6.40

u_{Rw}: Relative standard uncertainty due to within laboratory imprecision; RMS_{bias}: The mean square value of the monthly bias results of the laboratory; u_{cref}: the uncertainty of the reference value of the EQAS program.

dardization Program (NGSP) and the International Federation of Clinical Chemistry have taken actions for standardization of measurement [14, 15]. However, besides these studies and the effort of the societies, it is the responsibility of each clinical laboratory to ensure that HbA1c results meet appropriate analytical performance targets.

The analytical performance specification of HbA1c is recommended by the European Federation of Clinical Chemistry and Laboratory Medicine. This specification contains the laboratory's daily internal quality control's coefficient of variation at <2% and maximum acceptable total error (TE) of 6.7% [1]. According to current NGSP requirements for HbA1c assay, the within-run precision and total precision results should be ≤4% [16]. The precision values of our study meet the requirements of NGSP. The calculated TE was 6.08% in our study (calculated according to the formula: TEa=bias+1.65×CV); it was below the maximum acceptable TE. The precision, bias, and total allowable error parameters help laboratorians to see the analytical performance specification of the test results and take actions according to the results. However, the use of quality goals in clinical practice is limited.

The possible sources of uncertainty should be considered, such as the incomplete definition of the test, sample heterogeneity, uncertainty of calibrators, matrix differences between calibrators and samples, instability of the sample, reagents, and presence of interfering compounds in the sample [11]. The inclusion of measurement uncertainty in clinical laboratories increases the certainty of the measurement results. Also, it is recommended that MU should be included in reports of the test result [9]. Including MU in test reports is a requirement to ensure reliable, non-suspicious, clear, and understandable results in the decision-making process. MU can be presented as "test result±MU (number, percentage, or range)" [17, 18].

In our study, we evaluated MU for HbA1c results. HbA1c levels are important for DM management. The HbA1c level above 6.5% is one of the diagnostic criteria of DM. We estimated MU as 6.40% for HbA1c at the level of 6.5%. It can be presented as 6.5±0.4%. It is indicated that the MU of HbA1c should not exceed 0.5% at clinical decision levels [12, 19]. In a previous study, the measurement uncertainty of HbA1c was calculated to be 4.6% and was stated that the acceptable value was between 6.2% and 6.8% [20]. In a study evaluating measurement uncertainty of HbA1c levels on ion-exchange chromatography, the expanded uncertainty was calculated to be 7.4%. The author suggested that the results should be presented as 6±0.4% when considering the clinical decision level of HbA1c as 6%.

It can be said that presenting the measurement uncertainty is related to the evaluation of the laboratory analysis process rather than a clinical contribution. However, stating the measurement uncertainty with patient outcome reports may directly affect the clinical interpretation of the test result, especially for measurements at clinical decision levels. Clinicians are usually not aware of the variations of laboratory data or random errors. The knowledge of the uncertainty of an HbA1c measurement result would give the clinicians the necessary confidence to determine the certainty level of glycemic control of the patient, as this parameter helps in the interpretation of measurement results, especially when comparing a result with a decision limit.

One of the limitations of our study is the lack of reference material for bias estimation. We used external quality control materials, as it is practical.

To our knowledge, this is the first study evaluating the measurement uncertainty of HbA1c at Siemens Atellica Solutions.

In conclusion, it is important to report reliable test results for laboratorians as clinicians make their decisions based on laboratory data. We estimated the measurement uncertainty value of one of the most important tests in our laboratory. Our result meets the criteria. Laboratories should add measurement uncertainty results as a part of quality control programs. In particular, the laboratory tests with clinical decision limits should be presented with measurement uncertainty results.

Conflict of Interest: The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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