References


Component of Total Measurement Error for Hemoglobin A$_{1c}$ Determination, George Phillipov and Patrick J. Phillips (Endocrinology, The Queen Elizabeth Hospital, Woodville, South Australia 5011, Australia; * author for correspondence: fax 61-8-8222-6021, e-mail george.phillipov@nwahs.sa.gov.au)

The Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS), undertaken in people with type 1 and 2 diabetes, respectively (1,2), established the significance of glycohemoglobin (HbA$_{1c}$), and in particular hemoglobin A$_{1c}$ (HbA$_{1c}$), as a prognostic indicator for long-term micro- and macrovascular complications. However, the HbA$_{1c}$ measured during the DCCT and UKPDS represents a HbA$_{1c}$ fraction characterized by its retention time on cation-exchange HPLC rather than its unique chemical structure (deoxyfructosylhemoglobin). Accordingly, the DCCT HbA$_{1c}$ procedure represents a selective, but not a specific assay method (3).

Because no definitive or reference method exists for quantification of HbA$_{1c}$ (4), the American Diabetes Association (ADA), in collaboration with the Association of Clinical Chemists, implemented the National Glycohemoglobin Standardization Program (NGSP) (5) to standardize HbA$_{1c}$ values determined by methods different from that used in the DCCT. The ADA (6) now states that their recommended HbA$_{1c}$ thresholds, with respect to patient management goals, are valid only for NGSP-certified methods.

The variability of HbA$_{1c}$ measurements depends on both analytical and biological variation. However, because HbA$_{1c}$ concentrations are used for individual patient management, only analytical imprecision and within-person biological variation (s$_{I}^{2}$) are relevant. Whereas the NGSP (5) states that within-person HbA$_{1c}$ variance is negligible, previous studies (7,8) have reported s$_{I}^{2}$ estimates of 0.17–0.79, and investigations measuring HbA$_{1c}$ (9) or HbA$_{1c}$ (10) have reported values between 0.45 and 1.03. Accordingly, both analytical and within-person variability, but particularly the latter, increase measurement uncertainty and, therefore, the potential for clinical misinterpretation at ADA-specified HbA$_{1c}$ thresholds.

The following definitions are provided to avoid ambiguity with respect to terminology:

Within-person variance (s$_{I}^{2}$): the degree of random fluctuation of values around a person’s homeostatic set-point for a particular biological analyte. For people with diabetes, the HbA$_{1c}$ homeostatic set-point is controlled by dietary and/or pharmacologic treatment, and not by the normal physiologic mechanism. Repeatability (s$_{R}$): closeness of agreement between successive results obtained with the same method on identical test material and under the same conditions (same operator, same apparatus, same laboratory, and same time).

Reproducibility (s$_{P}$): closeness of agreement between individual results obtained with the same method on
identical test material but under different conditions (different operator, different apparatus, different laboratory, and/or different time). Analytical variance ($s_a^2$): comprises both within- ($s_w^2$) and between-assay components of variance. Total measurement variance ($s_m^2$): comprises both biological and analytical variance.

Serial HbA1c measurements were made in a cohort of 26 diabetic patients, in stable metabolic control, taking part in a 48-week multicenter trial (11). The mean number of specimens per patient was 7.2 (range, 6–9). All gHb determinations were performed with an affinity microcolumn assay (12), and results were converted to HbA1c percent-equivalents based on an algorithm originally derived by comparison (n = 186) with a HPLC method (13). During the trial period (2 years), $s_a$ for the affinity column method was 0.47 at a mean HbA1c concentration of 9.6%. The standard error of the estimate ($s_E$), calculated by nonparametric regression (HbA1c vs time) (14), was used to determine long-term variability associated with each patient’s serial HbA1c measurements. The corresponding mean $s_a$ was determined as the root mean square of the individual estimates (15).

Four different blood samples (~100 µL of each in a sealed ampoule), spanning HbA1c concentrations of ~6–13%, were hand-delivered on the same day to the five pathology laboratories performing physician-referred HbA1c assays in this State. The protocol (16) requires that all samples be analyzed in duplicate within a single analytical run. Three laboratories used Bio-Rad Variant HPLCs (NGSP-certified), and two used Pharmacia Mono S column HPLC systems (13) traceable to the Bio-Rad Diamat HPLC method. The cooperative trial method was defined as ion-exchange HPLC.

Routine data analysis was performed using SPSS for Windows, Release 10.0.7 (SPSS Inc.) and the Cbstat program (http://www.cbstat.com). The procedures described by Steiner (16) were used to calculate the corresponding estimates of repeatability and reproducibility for the interlaboratory study.

The individual estimates of $s_i$ were not statistically related to the respective baseline HbA1c concentrations (Kendall tau-b, 0.039; $P = 0.8$). The overall mean $s_m$ was 0.65, and given $s_m^2 = s_w^2 + s_a^2$, the within-person standard deviation $s_i = \sqrt{(s_m^2 - s_a^2)} = 0.44$.

For the interlaboratory study, the respective mean HbA1c values for the four samples were 5.8%, 7.8%, 10.4%, and 12.8%, and no laboratory showed consistently high or low HbA1c values, based on a method that ranks the sum of replicates. Similarly, no abnormal data were identified within the four samples when we used the Dixon test, and experimental variation between laboratories and between replicates was homogeneous. ANOVA established a significant variance ratio between laboratories ($F_{1,12} = 6.5; P < 0.05$) and for laboratory-sample interaction ($F_{12,20} = 30.7; P < 0.01$). Solving standard ANOVA equations (16), we calculated the between-laboratory ($s_L^2$) and laboratory-sample interaction ($s_{LS}^2$) variances as 0.143² and 0.12², respectively. The reproducibility ($s_r$), or variation arising from different operators, instruments, and laboratories is then given by:

$$s_r^2 = s_L^2 + s_{LS}^2 + s_o^2$$

and calculated as 0.19. Accordingly, among the five laboratories, 57% of total variance is between-laboratory, 40% is attributable to laboratory-sample interaction, and 3% is attributable to repeatability. The total error variance associated with the five HbA1c assays is therefore given by:

$$s_E^2 = s_r^2 + s_i^2$$

and is calculated as 0.48², of which $s_i^2$ contributes 84%. The design of the present cooperative trial, however, did not allow an estimate of long-term repeatability.

Knowledge of $s_a$ allows estimation of the range within which the true value lies at a reported HbA1c value, assuming that biological variability is that of a typical patient. Moreover, because $s_a^2 \ll s_i^2$, total error can be decreased more by analyzing additional specimens on the same patient than by performing more assays on the same specimen. This is highlighted in Table 1, which summarizes confidence ranges at different probabilities for analysis of one and two specimens.

**Table 1. Confidence ranges, at different probabilities, around a reported HbA1c concentration.**

<table>
<thead>
<tr>
<th>Probability, %</th>
<th>One specimen</th>
<th>Two specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>±0.94a</td>
<td>±0.71</td>
</tr>
<tr>
<td>90</td>
<td>±0.79</td>
<td>±0.60</td>
</tr>
<tr>
<td>80</td>
<td>±0.61</td>
<td>±0.47</td>
</tr>
<tr>
<td>60</td>
<td>±0.40</td>
<td>±0.31</td>
</tr>
</tbody>
</table>

*a* Implies that 95% of values should be within 0.94 above or below a reported HbA1c value.

Although the goal of the NGSP is to minimize bias between the DCCT and other HbA1c methods, and thereby allow uniform application of DCCT-derived HbA1c results, measurement uncertainty at ADA clinical decision-making thresholds has not been thoroughly addressed. In particular, failure to acknowledge the magnitude of within-person variation produces a significant underestimation of total measurement error. Our estimate of $s_i$ (0.44) is remarkably similar to the value of 0.41 reported previously by Hyltoft Petersen et al. (7), although both the experimental design and HbA1c methodology were different. In contrast, Kolatkar et al. (8)
reported lower values of 0.17 and 0.29 for 3- and 12-month study periods, respectively, where all patients were intensively treated and had HbA1c concentrations maintained at <7.0%.

Our findings for the five HPLC methods indicated that reproducibility was much less than within-person biological variation. However, the confidence intervals around a measured HbA1c concentration were still wide (Table 1) in comparison with the small difference (1%) between the ADA HbA1c management thresholds. The potential impact of measurement uncertainty on HbA1c thresholds has been discussed previously by Lytken Larsen et al. (17).

The most recently posted results for the College of American Pathologists HbA1c survey (18) show reproducibility values for certified methods between 0.19 and 0.85; the most common method (n = 335), the Abbott IMx (uncertified), had $s_i = 0.54$. Only HPLC methods had $s_i < 0.25$, whereas all multianalyte methods had $s_i \approx 0.43$. Minimal analytical performance has been proposed as 0.75$s_i$ (19), which based on our results is 0.33. However, the actual DCCT HbA1c procedure had a repeatability of 0.15 on masked split-duplicate specimens, whereas the long-term internal quality control showed a $s_i$ of $\sim 0.40$ (20).

In summary, the degree of within-person biological variation associated with HbA1c determinations significantly increases the total measurement error. If a HbA1c assay of high reproducibility is not used, the dispersion range of true HbA1c values around the mean true biological set-point will be so wide that the ADA management thresholds may become unworkable. Although the NGSP has significantly reduced intermethod bias, only some HPLC methods currently meet the required analytical performance (19).

We thank P. Charles, M. Haywood, Dr. M. Whiting, Dr. S. Sykes, and D. Moore for taking part in the HbA1c interlaboratory study.

References


Rapid and Sensitive Liquid Chromatography–Tandem Mass Spectrometry Method for Determination of Monoethylglycinexilidide, Frank Streit, Paul-Dieter Niedermann, Maria Shipkova, Victor William Armstrong, and Michael Oellerich (Department of Clinical Chemistry, George-August University Goettingen, Germany; * address correspondence to this author at: Abteilung Klinische Chemie, Zentrum Innere Medizin, Georg-August-Universität Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen, Germany; fax 49-551-398551, e-mail varmstro@med.uni-goettingen.de)

A large body of evidence currently documents the utility of the MEGX test for real-time assessment of liver function in transplantation, critical care medicine, and various experimental models (1). The test is based on the conversion of lidocaine to its deethylated metabolite monoethylglycinexilidide (MEGX), primarily through the hepatic cytochrome P450 system. In the standard MEGX test, an intravenous bolus of a small lidocaine dose (1 mg/kg) is administered over 2 min. Blood specimens are collected for serum MEGX determination both before and at 15 and/or 30 min after lidocaine administration. The most commonly used method to measure serum MEGX has been an automated fluorescence polarization immunoas-