Hemoglobin A1c Results and HbAS specimen

GH-01, GH-02, GH-03, GH-04, and GH-05 specimens were prepared from pooled whole blood obtained from healthy or diabetic individuals. The target values were determined from the means of all results from eight National Glycohemoglobin Standardization Program (NGSP) Secondary Reference Laboratories (SRLs). Each laboratory analyzed each specimen in triplicate on two separate days. These NGSP Network Laboratories use methods that are calibrated and traceable to the method used in the Diabetes Control and Complications Trial (DCCT). Comparison to the NGSP Network allows both manufacturers and clinical laboratories to trace their glycated hemoglobin results to the DCCT. The target HbA1c values for the Survey are as follows: GH-01, 5.46%; GH-02, 5.66%; GH-03, 9.31%; GH-04, 5.28%; GH-05, 7.41%.

The Survey evaluates results against the NGSP reference method targets with an acceptable limit equal to ± 6% of the target value. Because the proficiency testing (PT) specimens are prepared from human whole blood, the bias observed for the PT specimens is expected to reliably reflect the bias that exists for patient specimens analyzed with the same method.[1] The percentage is a mathematical fraction, not the HbA1c reporting unit. For example, the acceptable range for GH-01, which has a HbA1c value of 5.46%, would be HbA1c values between 5.1 and 5.8%.

In addition to the 6% grading criterion used for HbA1c, a second “dual grade” with an acceptable limit equal to ± 5% of the target value is shown on your laboratory evaluation. This second “dual grade” is provided for **educational purposes only** and is not reported to any laboratory accreditation agencies. Each laboratory must assess the accuracy and precision of its instrument, and if necessary, initiate appropriate actions.

For the five specimens, the pass rates vary considerably depending on the HbA1c method (data for all methods *n* ≥10 are summarized in Table 1). While the overall pass rate ranged from 87.1% (see below for discussion about GH-02) to 97.9%, depending on the target value, some methods were able to achieve 100% (or close to 100%) pass rates for all five specimens.

Table 1

Specimen	NGSP Target (% HbA1c)	Acceptable Range (+/- 6%)	Pass Rate % (Low/High)	Cumulative Pass Rate %
GH-01	5.46	5.1 - 5.8	80.0/100.0	94.0
GH-02	5.66	5.3 - 6.0	60.0/100.0	87.1
GH-03	9.31	8.7 - 9.9	86.7/100.0	96.0
GH-04	5.28	4.9 - 5.6	84.7/100.0	96.3
GH-05	7.41	6.9 - 7.9	89.6/100.0	97.9

Pass rates listed are for methods with a peer group *n* ≥ 10.

Examination of the HbA1c results obtained by participants in the Survey reveals that in general the mean values measured by the participants did not differ markedly from the values determined by the NGSP Secondary Reference Laboratories. The method-specific means for GH-05 (HbA1c

target value 7.41%) exhibited the least variation, ranging from 7.23% to 7.60% HbA1c (these are differences of -2.4 and +2.6%, respectively, from the target value). The method-specific means for GH-01 (HbA1c target value 5.46%) ranged from 5.18% to 5.71% HbA1c (differences of -5.1 and +4.6%, respectively, from the target value). GH-02 (HbA1c target value 5.66%) had method-specific means ranging from 5.43% to 5.74% HbA1c (differences of -4.1 and +1.4%, respectively, from the target value). (See below for additional information on GH-02.) GH-03 (HbA1c target value 9.31%) had method-specific means ranging from 8.91% to 9.55% HbA1c (differences of -4.3 and +2.6%, respectively, from the target value). GH-04 (HbA1c target value 5.28%) had method-specific means ranging from 4.99% to 5.41% HbA1c (differences of -5.5 and +2.5%, respectively, from the target value). Tosoh G8 Automated HPLC had CVs <1.5% for four specimens. Abbott Architect c System and Roche cobas c513 had CVs <2.0% for all five specimens. Guidelines from The National Academy of Clinical Biochemistry and the American Diabetes Association recommend an inter-laboratory CV <3.5% (Clin Chem 2011; 57:e1-e47 and Diabetes Care 2011; 34:e61-99). Most methods were able to achieve this criterion. However, Beckman AU Systems - Beckman reagent had CVs ≥3.5% for four specimens and Siemens Dimension ExL had CVs >3.5% for three specimens. Roche cobas c311 had the lowest mean value for three specimens.

In addition to the tables, the data obtained for each method (with a peer group $n \geq 10$) are also presented in the style of box-and-whisker plots (Fig. 1). Each method is listed individually, with the number of participants using that method in parentheses after the name of the method. The individual lines extend from the minimum to maximum difference, expressed as a percentage from the target value (the percentage is a mathematical fraction). The thicker line indicates the distribution of the middle 90% of values. The grey shaded area represents the evaluation limit, ie, ± 6% from the target. The diamond is the median for the particular method. Outliers were excluded. The presentation allows rapid visualization of bias [how far the diamond (median) is from zero], imprecision (length of the line) and the number of laboratories that failed (those that lie outside the shaded area) for each method. This feature provides additional detailed information that should be useful to individual laboratories to assess their method and compare it to both their peers and to other methods.

Hemoglobin variants may interfere with HbA1c assays (reviewed in *Clinical Chemistry* 2001; 45:153-163; also see http://www.ngsp.org). HbAS (sickle cell trait) is the most common hemoglobin variant in the US. Calculations based on disease prevalence suggest that HbAS is probably present in ~250,000 patients 18 years or older with type 2 diabetes in the US. In order to assess the effect of sickle cell trait on assays used in clinical laboratories, GH-02 was obtained from individuals with HbAS. Only those SRLs using methods that have no interference from HbAS determined the target value for GH-02. Excluding methods with interference from HbAS, GH-02 (HbA1c target value 5.66%) had method-specific means ranging from 5.43% to 5.74% HbA1c (differences of -4.1 and +1.4%, respectively, from the target value). Examination of the results for GH-02 show that the Bio-Rad D-10 and Bio-Rad Variant II Turbo 2.0, as well as all the Siemens methods, gave high values for the HbAS specimen. Laboratories that use these instruments and recovered outside the acceptable limits were assigned Code 30 (Scientific Committee decision) for GH-02. None of the other methods exhibited interference from the hemoglobin variant and results are consistent with the published literature (see http://www.ngsp.org).

Manufacturers of methods that have the means furthest from the reference value and those with the largest imprecision are encouraged to improve their performance, especially those methods that consistently exhibit large bias and/or large CVs. This is particularly important in the clinically relevant HbA1c ranges (~5.5% to 8%).

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