

Recent Progress in Glycohemoglobin (HbA_{1c}) Testing

The article by Schnedl et al. (1) addresses the interference of hemoglobin (Hb) variants in the measurement of glycohemoglobin (GHb). The degree of interference depends on both the specific Hb variant and the specific GHb method. Some methods identify the presence of a variant that may or may not interfere with results; other methods do not indicate the presence of a variant but may give inaccurate results.

Although GHb testing has been used for many years to monitor glycemic control in patients with diabetes, it was not until the completion of the Diabetes Control and Complications Trial (DCCT) (2) and the establishment of the National Glycohemoglobin Standardization Program (NGSP) (3) that GHb measurements could be used optimally.

The DCCT, which was completed in 1993, showed that the risks for development and progression of the chronic complications of diabetes are closely related to the degree of glycemic control, as measured by serial GHb determinations. The U.K. Prospective Diabetes Study (UKPDS), which was completed in 1998, showed results similar to those of the DCCT in patients with type 2 diabetes (4). Based on the DCCT results, the American Diabetes Association (ADA) now recommends that a primary treatment goal in diabetes should be blood glucose control at least equal to that achieved in the intensively treated cohort in the DCCT (5). They further recommend a specific GHb target (<7.0%) and action limit (>8%) based on the DCCT results (6). Thus, the DCCT results set the stage for establishing specific diabetes treatment goals that used GHb as an index of mean blood glucose values. However, when the DCCT ended in 1993, GHb test results were not standardized among laboratories, which prevented optimal clinical use of the test with respect to the new ADA recommendations.

Because of the positive impact standardization of GHb determinations would have on the care of diabetic patients, and in anticipation of the report of the DCCT results, the American Association for Clinical Chemistry (AACC) Standards Committee established a GHb standardization

subcommittee in April 1993. The goal of the subcommittee was to develop a plan for GHb standardization that would ultimately allow individual clinical laboratories to relate their GHb assay results to those obtained in the DCCT. Achieving this goal would have enabled individual clinical laboratories to provide diabetic patients and their health-care providers with test results that could be related directly to both mean blood glucose values and risks for development and/or progression of chronic diabetic complications.

Although the DCCT ended in 1993, the GHb assay systems from the study remained in place as part of another National Institutes of Health (NIH)-sponsored long-term diabetes study called the Epidemiology of Diabetes Interventions and Complications (EDIC). To initiate a standardization program in a timely fashion, the subcommittee recommended that the DCCT reference method be used as the reference or designated comparison method for GHb standardization while candidate definitive and reference methods and purified GHb standards continued to be developed and evaluated.

All GHb methods do not measure the same glyated component of hemoglobin. Boronate affinity methods separate "total GHb" according to structural characteristics of the glyated component. Immunoassay methods also rely on structural differences in Hb species, but they measure only the glycation on the NH₂-terminus of the β -chain (HbA_{1c} specifically). Ion-exchange and electrophoretic methods separate HbA_{1c} from other Hb species according to molecular charge. There was an excellent correlation in the results among these different types of methods, but without standardization of results to a common reference, reported results varied considerably (7). Because an important goal was to allow standardization of all types of assay methods, and given the wide variation in method types and analytes measured, the subcommittee proposed that standardization or calibration to the DCCT results could be performed best at the manufacturing level, where the most appropriate materials and standardization format for

each method could be determined. It was also proposed that verification of method standardization should be based on fresh sample comparisons with the DCCT reference method.

Once the development of a standardization protocol was completed, the subcommittee was dissolved, and the National Glycohemoglobin Standardization Program (NGSP) was organized to implement the protocol developed by the AACC subcommittee. Laboratories comprising the NGSP Reference Laboratory Network interact with manufacturers of GHb methods to assist them, first in standardizing their methods, and then in providing comparison data for certification of traceability to the DCCT. Whereas the standardization/calibration process is flexible and determined by the manufacturer, the certification process follows a specific protocol involving evaluation of both precision and accuracy by using specific criteria. As of September 1999, more than 25 different methods have been certified since the implementation of this program in July 1996. The College of American Pathologists (CAP) proficiency testing data, which use fresh blood samples and NGSP-assigned targets, have been used to evaluate the success of the NGSP.

Since the implementation of the NGSP, there have been considerable improvements in both the precision of GHb methods and comparability of results among methods, but there is still room for improvement. In 1999, compared with 1991, more laboratories are reporting their results in terms of HbA_{1c} or the equivalents of HbA_{1c} (78 vs. 34%, respectively), and ~42% of laboratories used an NGSP-certified method. CAP survey results have shown a clear improvement in comparability of results among laboratories. For most NGSP-certified methods, the 1999 method-specific medians were within 0.5% HbA_{1c} of the NGSP-assigned reference value. There is still significant between-laboratory variability with some methods, but for many methods, coefficients of variation are consistently below 5% (8).

In general, NGSP-certified methods have demonstrated less variability and better comparability to NGSP-assigned target

values than noncertified methods. Many of the methods certified clearly showed very high precision, low bias, and very little scatter in results, compared with the NGSP. There were, however, several methods that passed the NGSP certification criteria but showed a considerable amount of scatter around the regression line when compared with the results of an NGSP network lab. These results were observed even when these methods showed precision and bias within NGSP criteria. CAP survey data confirm that these methods show more between-laboratory variability and less comparability to the NGSP targets, making them less useful clinically compared with methods with less scatter. New certification criteria, which will be implemented in the last quarter of 1999, were developed to address this problem. Using the method of "assessing agreement," as described by Bland and Altman (9), the 95% CI of the differences between methods must fall within $\pm 1\%$ HbA_{1c}. The quality of NGSP-certified methods will continue to be evaluated with the introduction of these more stringent certification criteria.

DCCT traceable results are now being used on a national level as a means of measuring the quality of diabetes care (e.g., the ADA Provider Recognition Program and the Diabetes Quality Improvement Project); standardization of GHb is critical to this process. At the international level, the International Federation of Clinical Chemistry (IFCC) has developed a laboratory network that uses pure standards and specific HbA_{1c} reference methods. Efforts are underway to document the relationship between the IFCC and NGSP networks; understanding

this relationship could make DCCT traceable results available worldwide.

It should be noted that the NGSP has no specific guidelines or requirements for comparability for samples containing Hb variants. Samples with Hb variants and any other known potential interferences, such as carbamylated Hb, are specifically excluded from certification testing. These interferences are outside the realm of the NGSP certification process but should nonetheless be examined closely for each method. Hb variants are known to interfere with some methods; for other methods, the data are not so clear and need further evaluation. The degree of interference may vary with each new method and even with each method modification. When choosing a GHb assay method, a laboratory should select a method that is NGSP certified, as recommended by the ADA, and should consider its needs with regard to the patient population served (i.e., prevalence of Hb variants, kidney disease), test volume, turn-around time, level of expertise of laboratory staff, etc. Clinicians should insist that their laboratory's method is NGSP certified. They should also be aware of potential method interferences so that they can use their GHb results optimally, as recommended by the ADA.

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