Status of Hemoglobin A$_{1c}$ Measurement and Goals for Improvement: From Chaos to Order for Improving Diabetes Care

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BACKGROUND: The Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) established the importance of hemoglobin A$_{1c}$ (Hb A$_{1c}$) as a predictor of outcome in patients with diabetes mellitus. In 1994, the American Diabetes Association began recommending specific Hb A$_{1c}$ targets, but lack of comparability among assays limited the ability of clinicians to use these targets. The National Glycohemoglobin Standardization Program (NGSP) was implemented in 1996 to standardize Hb A$_{1c}$ results to those of the DCCT/UKPDS.

CONTENT: The NGSP certifies manufacturers of Hb A$_{1c}$ methods as traceable to the DCCT. The certification criteria have been tightened over time and the NGSP has worked with the College of American Pathologists in tightening proficiency-testing requirements. As a result, variability of Hb A$_{1c}$ results among clinical laboratories has been considerably reduced. The IFCC has developed a reference system for Hb A$_{1c}$ that facilitates metrological traceability to a higher order. The NGSP maintains traceability to the IFCC network via ongoing sample comparisons. There has been controversy over whether to report Hb A$_{1c}$ results in IFCC or NGSP units, or as estimated average glucose. Individual countries are making this decision.

SUMMARY: Variability among Hb A$_{1c}$ results has been greatly reduced. Not all countries will report Hb A$_{1c}$ in the same units, but there are established equations that enable conversion between different units. Hb A$_{1c}$ is now recommended for diagnosing diabetes, further accentuating the need for optimal assay performance. The NGSP will continue efforts to improve Hb A$_{1c}$ testing to ensure that clinical needs are met.

The importance of glycated hemoglobin (GHB) or hemoglobin A$_{1c}$ (Hb A$_{1c}$) as a marker of glycemic control in diabetes mellitus was emphasized by the results of the Diabetes Control and Complications Trial (DCCT), and its continuation as the Epidemiology of Diabetes Intervention and Complications (EDIC) (1, 2), as well as by the United Kingdom Prospective Diabetes Study (UKPDS) (3). The results of these 2 prospective long-term randomized studies conclusively demonstrated that intensive glycemic control significantly reduces the risk of long-term microvascular complications of both type 1 and type 2 diabetes and allowed the establishment of specific treatment goals based on Hb A$_{1c}$. On the basis of the Hb A$_{1c}$ results from these trials, most clinical diabetes organizations worldwide now recommend either 6.5% or 7% Hb A$_{1c}$ as a target for glycemic control in the majority of patients with diabetes (4, 5).

When the DCCT ended in 1993 there were considerable differences in how GHB results were reported. The 1993 College of American Pathologists (CAP) proficiency survey revealed that only 50% of participating laboratories were reporting results as Hb A$_{1c}$, whereas 21% were reporting results as Hb A$_{1}$ and the remaining 29% were reporting results as total GHB (6). There was also substantial variability even within each reporting category. This variability among GHB values made it very difficult for physicians to use specific Hb A$_{1c}$ targets in clinical practice because the results they ob-

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3 Nonstandard abbreviations: GHB, glycated hemoglobin; Hb A$_{1c}$, hemoglobin A$_{1c}$; DCCT, Diabetes Control and Complications Trial, UKPDS; EDIC, Epidemiology of Diabetes Intervention and Complications; UKPDS, United Kingdom Prospective Diabetes Study; CAP, College of American Pathologists; NGSP, National Glycohemoglobin Standardization Program; CPRL, central primary reference laboratory; PRL, primary reference laboratory; SRL, secondary reference laboratory; ADA, American Diabetes Association; eAG, estimated average glucose; POC, point-of-care; RCV, reference change value.
tained were, in many cases, not directly related to those of the clinical studies from which the targets were derived. This problem of variability among results was avoided during the DCCT by having all analyses performed in a single laboratory that reported Hb A1c.

In 1993 the AACC developed a subcommittee for the standardization of Hb A1c measurement, and in 1996 the National Glycohemoglobin Standardization Program (NGSP) was initiated to implement the AACC protocol (7). The goal of the program was to standardize Hb A1c test results so that clinical laboratory results are comparable to those reported in the DCCT and UKPDS, where relationships to risk for microvascular complications had been established. Similarly, Hb A1c standardization programs were initiated in Sweden and Japan in the mid 1990s (8, 9). In this review we discuss the improvements in Hb A1c measurements that are the result of NGSP activities. We also describe the current status of Hb A1c testing and address plans for improvements in the future that will ensure that the quality of Hb A1c testing meets clinical needs.

The NGSP Network

The NGSP network and processes are shown schematically in Fig. 1 and are also described in detail on the NGSP Web site (http://www.ngsp.org). The NGSP network consists of the central primary reference laboratory (CPRL), 3 primary reference laboratories (PRLs), and 7 secondary reference laboratories (SRLs). PRLs and SRLs are located both in Europe and the US. The CPRL method is the DCCT/EDIC Bio-Rex 70 HPLC reference method. The PRLs, which use the same method as the CPRL, function as backup laboratories. The SRLs use highly precise commercial methods based on ion-exchange HPLC, immunoassay, boronate-affinity HPLC, or capillary electrophoresis. NGSP network laboratories are monitored monthly with 10 pooled frozen whole blood samples spanning the range of 4%–10% (4%–12% before January 2010) Hb A1c, and all network laboratories are compared to the CPRL. In addition, comparisons with the IFCC network are performed twice per year. To pass the NGSP-monitoring exercise, the estimate of the SD of the difference between sample replicates (each sample is analyzed on 2 separate days) must not exceed 0.229% Hb A1c. In addition, the mean of the differences between an individual network laboratory and the CPRL must not exceed 0.35% Hb A1c. SRL results must also fall within a defined acceptance ellipse based on the slope and intercept of the differences between the results of the individual SRL and the medians of all SRLs. An example of a monthly SRL report including a precision chart, bias charts, and an acceptance ellipse is shown in Fig. 2. Moreover, results for single samples for which the within-SRL replicates differ by >0.4% Hb A1c or the mean result differs from the median of the SRLs by >0.5% Hb A1c are excluded from the final mean SRL calculations. All monitoring results are shared among the network laboratories. If an SRL fails a monitoring exercise, the laboratory is prohibited from participating in certification until the problems are addressed and the method passes the next monitoring exercise. The mean between-laboratory CVs for the NGSP network for each month between August 2009 and July 2010 are shown in Fig. 3. All monthly network CVs were below 2.5%. NGSP network values are also directly traceable to the IFCC laboratory network that performs the “higher order” reference measurement procedure for Hb A1c (see section below).

The NGSP Process

The 3 major processes of the NGSP are shown in Fig. 1. NGSP network laboratories assist manufacturers with the calibration of their methods. Once calibrated, these methods can be certified. The certification process consists of an exchange of 40 fresh or frozen whole blood samples between a manufacturer and an NGSP SRL.
Manufacturers can choose the SRL to which they want to compare when they certify their methods. The choice may be based on location or method type. In some situations, the variability among individual results is lower when similar method types are compared, e.g., when both the manufacturer and the SRL use an ion-exchange HPLC method. A manufacturer is awarded a Certificate of Traceability if the 95% CI of the differences between their method and the SRL falls within \( \pm 0.75\% \) Hb A\textsubscript{1c} (Table 1). Each certificate is effective for 1 year from the date of certification. To maintain continuous certification, the certification process must be repeated annually \((7, 10)\).

Individual laboratories can also be certified if they choose. Laboratories involved in clinical trials are most likely to be certified at the request of the clinical trial sponsor. Other laboratories with high Hb A\textsubscript{1c} sample volume may also be interested in certification. A relatively small fraction (approximately 1%–3%) of accredited laboratories in the US are NGSP certified. The certification process for laboratories is the same as that for manufacturers, but there are 2 levels of laboratory certification. Level II certification criteria are the same as those for manufacturers. Level I certification is tighter; the 95% CI of the differences between methods (laboratory compared to SRL) must fall within \( \pm 0.70\% \) Hb A\textsubscript{1c} (Table 1). Level I laboratories, unlike level II, are monitored quarterly by the same process and criteria used for NGSP network laboratories.

The third component of the NGSP process is surveillance of the CAP Hb A\textsubscript{1c} proficiency data. This process is critical for monitoring the success of the NGSP.
(see “From Chaos to Order” below). The CAP GH2 survey is performed twice a year. Fresh whole blood samples are used and grading is accuracy based, with target values assigned by the NGSP. Gradual tightening of the CAP criteria has added momentum for laboratories and manufacturers to improve the quality of Hb A1c testing.

There has been a steady increase in the number of methods and laboratories that have been certified since the NGSP was initiated in 1996. In mid 2010 there were approximately 100 methods and 100 laboratories certified. Of the certified laboratories, approximately 85% were level I, and 75% were located outside the US. This progressive increase in certification documents the ability of manufacturers to continuously improve their methods. The growth in both manufacturer and laboratory certifications also reflects continued demand for methods and laboratories that can meet the needs of diabetes care teams, clinical research, and clinical trials. A list of NGSP-certified methods and laboratories (updated monthly) is available on the NGSP Web site (11).

The IFCC Network

The primary goal of the IFCC working group on Hb A1c standardization, initiated in 1995, was the development of an internationally accepted higher order reference method and reference materials for Hb A1c measurement. The reason for developing this method was to satisfy concerns of traceability to a “higher order method,” a need that was promulgated by the European Union In-Vitro Diagnostic directive of 1998 (12). Although the Bio-Rex 70 method used throughout the DCCT was shown to be very consistent throughout the DCCT (1993–2003) and continuing through the EDIC (1993–2010) (13), this method is considered a “designated comparison method” and not a higher order method because it is not completely specific for Hb A1c and does not use pure standards. The IFCC Reference Method for Hb A1c was approved in 2001 (14). The IFCC has established a laboratory network that comprised 11 laboratories at the time of this report. Each laboratory runs 1 or both of the 2 approved IFCC methods, namely HPLC–mass spectrometry or HPLC–capillary electrophoresis (15). Each method gives essentially identical results because they use the same primary reference materials for calibration. It is important to emphasize that neither the NGSP CPRL method nor the IFCC method is suitable for routine measurement of Hb A1c in patient samples.

The IFCC offers manufacturers matrix-appropriate calibrators and controls with values assigned by the IFCC network to establish and check their traceability, as well as a monitoring program that facilitates ongoing monitoring of their traceability status on a bimonthly basis (16). However, there is no certification program. Multiple comparisons between the networks reveal that results obtained by the IFCC method are highly correlated with NGSP/DCCT results, but there is a bias. This bias was translated into a “master equation” that describes the linear relationship between results from the IFCC and NGSP laboratory networks: NGSP = (0.915 × IFCC) + 2.15 (17). The master equation, developed from 4 internetwork IFCC/NGSP comparison studies over a 2-year period, is monitored regularly via ongoing internetwork studies to insure consistency of the relationship; thus far the relationship has proven stable for more than 7 years (15). However, implementation of the IFCC Reference System created a problem as to how Hb A1c results should be reported, because there are different interpretations of “traceability.” One interpretation is that all clinical results must be reported in IFCC method numbers and units. By contrast, others believe that changing the reported scale of numbers and the units confuses healthcare providers and patients and should not be done, and insuring that NGSP numbers can be related back to the higher order method is sufficient to fulfill traceability requirements.

THE NGSP AND IFCC: WHAT NUMBERS AND UNITS SHOULD BE REPORTED?

In an effort to find some compromise, 3 major clinical diabetes organizations—the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), and the International Diabetes Federation—agreed that laboratories should report an estimated average glucose (eAG) (18). After a large prospective international study to determine the relationship between Hb A1c and mean blood glucose, a linear relationship was established (19). Notwith-
standing these findings, many experts believe there was too much variability in the Hb A1c/mean blood glucose relationship and the eAG should not be reported.

A consensus statement on the worldwide standardization of Hb A1c was published in 2007 (20) and was revised in 2010 (21). According to the 2010 statement, Hb A1c should be reported in both DCCT and NGSP units, namely %Hb A1c and millimoles per mole. Despite this official consensus, individual countries are deciding the actual method of reporting Hb A1c. Several countries have already decided to switch to reporting IFCC numbers only, usually after an interim period of reporting both IFCC and NGSP numbers, but many countries have not yet determined how they will report Hb A1c. The US has decided to report NGSP numbers (i.e., %Hb A1c) and eAG, but not IFCC numbers (mmol/mol). The use of eAG has not been accepted outside the US. Despite the lack of global consensus for reporting Hb A1c, there is now a clear way to relate the different reported results. For example, with the use of the master equation, it is easy to convert from IFCC to NGSP numbers and vice versa. Many instruments that measure Hb A1c will be able to provide Hb A1c results in NGSP or IFCC units or both units, and most laboratories have the option of also reporting eAG (along with Hb A1c) through their laboratory information systems. It is important to emphasize that the documented relationship (master equation) between NGSP and IFCC continues to be monitored. The continuous monitoring within and between networks provides added security for the stability of reported Hb A1c results, regardless of which units are used.

**Improvement in the Measurement of Hb A1c**

The NGSP and IFCC approaches to the standardization of Hb A1c results serve different—and complementary—purposes. The primary objective of IFCC standardization is to ensure that manufacturers of Hb A1c assay methods are traceable to an accuracy base. Each manufacturer must document traceability to the IFCC higher order method through an unbroken traceability chain, and the uncertainty of each step must be documented. However, the IFCC network does not set a limit on the degree of uncertainty allowed between a manufacturer’s method and the IFCC value. The NGSP standardization program has defined acceptable limits for method performance that are based on clinical requirements.

The NGSP certification process comprises analysis in duplicate of a panel of 40 samples measured by both the manufacturer and one of the NGSP SRLs over a period of at least 5 days (e.g., 8 samples per day in duplicate on each of 5 days) at both sites. The NGSP certification criteria were tightened in 1999, 2002, and 2007, and again in January 2010. The current requirements are that the Hb A1c concentrations of the 40 samples be between 4% and 10% Hb A1c, with specific numbers of samples in defined ranges. The 95% CI of the differences between the certifying method and the SRL must fall within ±0.75% Hb A1c for manufacturer certification. This Bland–Altman assessment of agreement (22) is an estimate of total error, for which both the bias and variability of the method are taken into account. Manufacturers must demonstrate that their method meets these requirements for it to be certified by the NGSP as traceable to the DCCT. By gradually and progressively tightening certification limits (23) the NGSP has brought about improvement in the accuracy and precision of Hb A1c measurements.

In addition, the NGSP has worked with CAP in the adoption and subsequent tightening of accuracy-based grading by using NGSP targets for the CAP GH2 whole-blood proficiency survey. The CAP provides the largest proficiency-testing survey for Hb A1c worldwide. Current (2010) enrollment exceeds 3000 laboratories, many located outside the US. Since its inception, the NGSP Steering Committee has worked closely with the CAP to insure that proficiency data for Hb A1c are optimal for assessing the state of the art in Hb A1c testing and also to encourage better assay performance. In 1996, the CAP began using pooled fresh whole blood specimens for the GH2 survey. Because the samples are free of the matrix effects that plagued previous Hb A1c surveys, results could reliably be used to examine bias and imprecision within and among methods. In 2005 the CAP began providing a “dual grade” for educational purposes, which permitted laboratories to compare their results to the NGSP target values. Two years later accuracy-based grading was adopted as the exclusive criterion for the CAP Hb A1c surveys. The initial acceptable limit for accuracy grading was set at ±15%. This value was progressively lowered in 2008, 2009, and 2010 to ±12%, ±10%, and ±8%, respectively. In 2011 the limit will be ±7%.

**From Chaos to Order**

The NGSP has tracked the progress of Hb A1c standardization since 1996 via assessment of CAP survey results. In 1993, when the DCCT ended and the ADA began recommending specific Hb A1c goals for patients, laboratories reported different glycated fractions (Hb A1c, Hb A1s, or total GHb) and there was substantial variability among results (Fig. 4, left panel). By 1999, 3 years after the NGSP was established, 80% of laboratories were reporting GHb results as Hb A1c. By 2004 almost all laboratories were reporting results as Hb A1c, and there was considerably less variability both within and between methods (Fig. 4). Beginning in 2009, only Hb A1c results were accepted on the CAP survey. Im-
Improvement in method performance between 2000 and 2010 is shown in Fig. 5. The mean, SD, and CV for all results combined are calculated in each survey. The all-method CV decreased from approximately 7% to 4.0% between 2000 and 2010 in the low (nondiabetic) range samples (4%–6% Hb A1c) (Fig. 5C). The improvement over time has been more modest for samples in the 6%–10% Hb A1c range, with all-method CVs decreasing from approximately 5%–5.5% to approximately 4% (Fig. 5, B and C). Many methods now have between-laboratory CVs ≤3%, and a few are ≤2%. HPLC methods generally demonstrate better precision than immunoassay methods but there is some overlap in the CVs of the 2 method types. In the GH2 2010A survey the between-laboratory CVs for immunoassays ranged from 1.7% to 6%, whereas for HPLC methods the CVs were 1.4%–3.5%. At the time of this report, 11 point-of-care (POC) devices were NGSP certified. Very little proficiency data are available for POC methods, but at least 1 POC device performs as well as many of the laboratory methods in the CAP survey (24).

Each survey participant was graded on the basis of the NGSP value assignment, with passing set at ±8% in 2010. Each participant was also given an “educational grade,” which was at the projected pass limit of ±6%. At the current ±8% acceptable limit, the pass rates (i.e., percentage of laboratories passing) for the 2010A GH2 survey for all laboratories for each specimen were more than 95% (24). Although the pass rates were lower at the more stringent ±6% limit, as expected, the overall laboratory pass rate for each sample was still close to 90% (91.0%, 88.6%, and 91.6% for the low, medium, and high Hb A1c concentrations, respectively).

Note that the CAP limits, which are based on a percent of the target for each individual survey sample, are not readily comparable to the NGSP certification process, which is based on a 95% CI for 80 (40 samples run in duplicate) results. Clearly the NGSP limits will need to be tightened to “match” the 2011 CAP limit of 7%. Nevertheless, the most recent NGSP certification data show that for the 15 most widely used methods on the 2010A CAP survey, 90% of certification results are within ±7% of the SRL. Eleven of these 15 methods had ≥95% of results within ±7%.

Analytical Goals for Hb A1c

The DCCT demonstrated significant differences in outcome risks between the intensive and standard treatment groups, for which the mean Hb A1c concentrations were only approximately 2% Hb A1c apart (7.2% vs 9.1% Hb A1c, respectively) (1). Similarly, despite an even lower difference in mean Hb A1c values (7% vs 7.9% for the intensive and conventionally treated groups, respectively), the UKPDS revealed a significant reduction of microvascular complications in intensively treated patients with type 2 diabetes (3). It is therefore essential that Hb A1c be measured with precision and accuracy that enables healthcare providers to reliably distinguish optimal from suboptimal glycemic control. The recent recommendation to use Hb A1c for the diagnosis of diabetes (4, 25) further emphasizes the need for optimal assay performance. The ADA now recommends that Hb A1c can be used for diagnosis of diabetes with a threshold of ≥6.5% (4).
Many believe that performance goals for Hb A\textsubscript{1c} testing should be based on clinical requirements. There are 2 important questions for a physician to consider when using Hb A\textsubscript{1c} measurements to assess a patient’s glycemic status. One is whether the patient’s glycemic control is stable, improving, or deteriorating, and the other is how the Hb A\textsubscript{1c} result compares to the individual’s target Hb A\textsubscript{1c} (the current ADA recommendation is 7% with the qualification that lower is better if feasible). In answer to the first question, many physicians have suggested that 0.5% Hb A\textsubscript{1c} is a “clinically significant change.” Importantly, treatment guidelines and algorithms from the ADA/EASD (26) and National Institute for Clinical Excellence in the UK (27) recommend evaluating new treatment regimens in terms of whether Hb A\textsubscript{1c} is lowered by 0.5 percentage points or more. In answer to the first question, many physicians have suggested that 0.5% Hb A\textsubscript{1c} is a “clinically significant change.” Importantly, treatment guidelines and algorithms from the ADA/EASD (26) and National Institute for Clinical Excellence in the UK (27) recommend evaluating new treatment regimens in terms of whether Hb A\textsubscript{1c} is lowered by 0.5 percentage points or more. Therefore it is important to be sure that a change of this magnitude is statistically significant and not due to analytical variation. Taking a statistically significant difference of 0.5% Hb A\textsubscript{1c} at an Hb A\textsubscript{1c} concentration of 7% as the goal for Hb A\textsubscript{1c} measurement, one can use

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\text{RCV} = 0.5\% \text{Hb A}\textsubscript{1c} (0.43\%) \quad \text{(see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue2)}.
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For sequential results to be significantly different, the numbers must differ by more than the combined variation inherent in the 2 results: RCV (\%) = \(2^{(1/2)} \times 1.96 \times \left[ (CVA)^2 + (CVI)^2 \right]^{1/2} \), where CVA is the analytical CV of the method (within-laboratory CV) and CVI is the within-subject biological variation. For Hb A\textsubscript{1c} the CVI is low, \(<1\% \) (29), when estimated in individuals without diabetes. If the analytical CV of the Hb A\textsubscript{1c} method is 2% (feasible for many commercially available HPLC systems), then the RCV (95% probability) is \(<0.5\% \text{Hb A}\textsubscript{1c} (0.43\%) \) (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue2). Approximately 80% of laboratories participating in the 2010 CAP GH2 survey were using methods that were capable of within-laboratory CVs \(\leq 2\% \). We can conclude that, at least when Hb A\textsubscript{1c} is measured in an accredited laboratory, physicians can be reasonably (95%) certain that a difference of 0.5% Hb A\textsubscript{1c} or
greater between successive patient samples represents a statistically significant change in glycemic control. If the laboratory is using a method that demonstrates a within-laboratory CV >2%, as is still the case with some assay methods, there is less confidence that a difference of 0.5% Hb A1c is significant (online Supplemental Table 1).

In the situation in which a physician wants to look at the difference between a patient result and a goal of 7% Hb A1c, both the bias and variability (%CV), in other words the total error, of the method must be taken into account. For example, if a method has 0 bias, a CV of 3.5% is required to have 95% confidence that the Hb A1c result for a patient with a “true” result of 7% will read between 6.5 and 7.5% (±7%). If there is a bias of 0.2% Hb A1c, the CV requirement would tighten to 2.3% (online Supplemental Table 2). If a narrower range for this degree of certainty is required (e.g., 95% confidence that the value will be within 6.7%–7.3% Hb A1c), the analytical CVs required are even lower. Where CAP grading is concerned, the current limit of ±8% corresponds to a limit of ±0.56% Hb A1c at a target value of 7% Hb A1c. The ±7% limit to be implemented in 2011 corresponds to ±0.49% Hb A1c. For all laboratories to give results that are within ±0.5% Hb A1c, either at the 7% Hb A1c ADA goal, or at the diagnostic threshold of 6.5% Hb A1c (4), then a CAP cutoff of ±7% is appropriate. More than 90% of laboratories participating in the most recent CAP survey would pass at a ±7% cutoff. At Hb A1c values above 7%, the CAP cutoff would have to be tighter to be within ±0.5% Hb A1c.

Interferences with Hb A1c Testing

For the vast majority of patients with diabetes, Hb A1c provides an excellent measure of glycemic control. Nevertheless, there are situations in which Hb A1c results may be unreliable. These include circumstances that involve factors that interfere with the actual measurement of Hb A1c, such as certain hemoglobin variants or adducts (30–34), as well as factors that affect the interpretation of Hb A1c results. The latter includes severe iron-deficiency anemia or any condition that alters the erythrocyte lifespan (e.g., hemolytic anemia, renal failure) (35–39). Additional factors, such as race and age, have also been reported to influence Hb A1c. Data reveal that Hb A1c increases by approximately 0.1% with every 10 years of age (40, 41). Nevertheless, this small increase is unlikely to necessitate a change in treatment goals for different age groups. Hb A1c has been shown to be higher in certain ethnic groups, but ethnic differences in the relationship between Hb A1c and mean blood glucose remains a controversial topic (42–47).

Generally, individuals heterozygous for hemoglobin variants or with increased concentrations of Hb F do not have shortened erythrocyte survival, and Hb A1c can be measured accurately if an appropriate assay method is used. Several reports have included analysis of the effects of these hemoglobins on Hb A1c results (31–34). The findings are summarized on the NGSP Web site (48). These interferences are usually method specific. In general, Hb AS and Hb AC (2 of the most common variants in the world) interfere with some immunoassays, although the most widely used immunoassays are now free of this interference. Hb AE and Hb AD interfere with some HPLC methods, but do not affect immunoassays, most likely because of the distance between the N-terminal Hb A1c modification site and the chain. If an ion-exchange HPLC method is used, careful inspection of chromatograms usually reveals aberrant peaks produced by most variants, enabling detection of unacceptable results. As with any test, results that are inconsistent with the clinical presentation should be investigated further. Hb F <10% does not affect most methods. However, samples with Hb F >10% need to be analyzed by methods that are free from Hb F interference to obtain accurate results.

Summary

Considerable progress has been achieved in the standardization of GHb results since the implementation of the NGSP in 1996. Virtually all laboratories in the US, and in many other countries, now report results as Hb A1c (IFCC, NGSP, eAG, or some combination) and overall variability has been considerably reduced.

The IFCC has established a reference method and materials that enable documentation of traceability to a higher order reference. Although the numbers used to report Hb A1c (IFCC, NGSP, eAG, or some combination) are being decided on a country-by-country basis, established equations permit ready conversion among these numbers. The NGSP and IFCC networks have complementary roles and maintain close interactions.

Despite the progress that has been made, reduction in total error for some methods is still needed given the importance of Hb A1c for monitoring therapy and the recent recommendation to use Hb A1c for diagnosis of diabetes (4). Further reductions in the NGSP and CAP acceptable limits are likely in the future, and ongoing monitoring of the impact of these measures on the quality of Hb A1c results for patient samples will continue, with the goal of ensuring that the quality of Hb A1c testing is sufficient to meet clinical needs.

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