A 14 year-old African-American boy living in the southeastern United States presented to the emergency department (ED) with his parents in the late evening with complaints of chronic thirst, occasional vomiting, fatigue, and frequent urination. These symptoms had all increased in severity over the past several weeks. The patient’s ED physician ordered a STAT laboratory test panel, which indicated hyperglycemia and diabetic ketoacidosis (see Table 1). According to the American Diabetes Association (ADA) guidelines, the boy suffered from diabetes due to the presence of unequivocal hyperglycemia with acute metabolic decompensation (refer to References 1 and 2 for a current review of the diagnosis of diabetes). The patient was, ultimately, diagnosed with type I diabetes and, subsequently, admitted to the hospital where he began insulin therapy and nutritional counsel. Thereafter, self-monitoring of glucose and a three-month follow-up visit with an endocrinologist, along with HbA1c testing, were recommended.

The compliant patient provided his previous three-month self-monitoring glucose values to the endocrinologist upon his first visit. In addition, the patient’s result for the HbA1c test performed by a hospital laboratory two days prior to his visit was ready for the endocrinologist to review. Based on the patient’s self-monitored glucose values (performed six to seven times daily), for the past three months his average blood glucose was calculated as 205 mg/dL (11.5 mmol/L). The HbA1c value for this patient, however, measured only two days prior was reported as 6.5%, reflecting a mean blood glucose for the past two to three months of ~153 mg/dL (8.6 mmol/L) — contrary to what was observed using the patient’s self-monitoring values.

The endocrinologist confirmed the HbA1c value using a different specimen drawn on the day of the patient’s endocrinology visit. The discrepancy observed between the patient’s HbA1c and mean blood-glucose values was determined not to be the result of hypoglycemic episodes between glucose measurements or undetected nocturnal hypoglycemia. This prompted the boy’s physician to investigate the presence of an underlying disease possibly causing a reduced red-blood-cell (RBC) survival, resulting in a falsely decreased HbA1c value. Notably, an Hb electrophoresis test performed on the boy indicated the presence of an HbC variant, an abnormal Hb associated with a reduced RBC life span.

The purpose of this article is to provide a review of the implications of diabetes and HbA1c, standardization efforts of
HbA1c for use as a long-term monitor of average glycemia, the pathobiology of HbA1c, as well as current measurement pitfalls associated with clinical-laboratory measurements.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose</td>
<td>410 mg/dL (22.78 mmol/L)</td>
<td>60 mg/dL to 100 mg/dL (3.33 mmol/L to 5.56 mmol/L)</td>
</tr>
<tr>
<td>Urine ketones</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Serum ketones</td>
<td>Positive (1:8)</td>
<td>Negative</td>
</tr>
<tr>
<td>Anion gap</td>
<td>18 mmol/L</td>
<td>3 mmol/L to 11 mmol/L</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.28</td>
<td>7.35 to 7.45</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>17 mmol/L</td>
<td>24 mmol/L to 32 mmol/L</td>
</tr>
</tbody>
</table>

**Table 1.** Patient laboratory results

**Background of diabetes and HbA1c**

Diabetes is currently the seventh leading cause of death in the developed world.3 In the United States, this devastating disease accounts for more than $132 billion in healthcare costs annually and these costs are predicted to rise as high as $192 billion by the year 2020 (see Figure 1).4,5,6 According to the ADA, diabetes mellitus is defined as “a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.”1 The disease causes high levels of blood glucose as a result of defects in insulin production (type I), insulin action (type II), or both.7,8,9,10 This, in turn, often leads to long-term medical complications, which compound the costs of this devastating disease.4,11

![Economic cost of diabetes in the United States](image)

**Figure 1.** Economic costs (both direct and indirect) of diabetes in the United States. Adapted from References 1, 2, and 3.

Uncontrolled glucose concentrations in diabetic patients can lead to serious medical complications and premature death.7,10,11,12,13 Previous research trial groups, including the Diabetes Control and Complications Trial (DCCT) conducted from 1983 to 1993, and the U.K. Prospective Diabetes Study (UKPDS), the largest clinical research study on diabetes initiated in 1990, both demonstrate a strong relationship between the level of plasma-glucose control for both type I and type II diabetes and the risk of late retinal, renal, and neurological complications.11,13 The DCCT study further showed that type I diabetics who maintain average plasma-glucose concentrations closer to the normal range exhibit a significantly lower incidence of microvascular complications,11 while the UKPDS study provided conclusive evidence that the life-threatening complications of type II diabetes also can be significantly reduced by appropriate treatment, such as lowering mean blood-glucose levels.13

Results from these large, prospective, randomized clinical trials demonstrated that intensive treatment with the goal of normal glycemic levels (or as close to normal as possible), decreases the frequency and severity of diabetic complications.11,13 Importantly, although there is some evidence linking hyperglycemia to adverse patient outcome, substantially more data are available that directly correlate increased HbA1c with complications of diabetes. Analyses of the DCCT and UKPDS trial outcomes linked HbA1c measurements to average blood-glucose control vs. individual plasma-glucose concentrations. Based on the direct relationship between HbA1c and diabetic complications, recent guidelines for the management of diabetes now stress the importance of monitoring HbA1c.1,2,14,15 Measurement of HbA1c, as an estimate of long-term average glycemia, assists diabetics as well as their physicians by providing treatment goals to reduce the risks associated with the development and progression of chronic complications of diabetes.2,12,14,15

The DCCT showed a strong correlation between HbA1c and mean blood glucose, such that an HbA1c value of 7% represented a mean blood-glucose value of approximately 170 mg/dL (8.3 mmol/L), and an HbA1c value of 9% represented a mean blood-glucose value of about 240 mg/dL (11.7 mmol/L).11 Additionally, the DCCT and UKPDS trials led the ADA to recommend that a primary treatment goal in adults with diabetes should be near-normal glycemia with an HbA1c <7% when measured by the same method used in the DCCT (cation-exchange HPLC [high-performance liquid chromatography]).1,14,15 Not only was the significance of using a particular method to measure HbA1c values in diabetics emphasized as a result of the DCCT and UKPDS trials, but also there was the realization that the various methods used around that time produced clinically significant variability.15 As a result, efforts to standardize the measurement of HbA1c across all clinical laboratories were needed to aid in the management and monitoring of patients with diabetes, which led to the formation of the National Glycohemoglobin Standardization Program (NGSP) in 1996.

**National HbA1c standardization efforts**

The importance of standardizing HbA1c methods became increasingly apparent after the completion of the DCCT in 1993.17 Because of the positive impact standardization of HbA1c determinations would have on the care of diabetic individuals, the ADA was instrumental in the formation of the NGSP. The NGSP determined that the HbA1c determinations used in the clinical laboratories were not standardized, and efforts were needed to ensure that the results obtained from different laboratories were comparable.

Continues on page 12
patients as seen by the study, the American Association for Clinical Chemistry (AACC) Standards Committee established a National Glycated Hb Standardization Subcommittee in April 1993, currently referred to as the NGSP. At the time the results from the DCCT were published, various methods were used in clinical laboratories to measure HbA1c, and no reference method was in place to standardize testing. This led to con-

Figure 2A. Formation of glycated hemoglobin in normoglycemia (A) and hyperglycemia (B) conditions. 1A&B) RBCs produced in the bone marrow enter and fully mature in the circulation (Day zero). In a normal person, RBCs have an average life span of ~120 days (~Day 120). The RBCs are freely permeable to glucose and the concentration in the cell is approximately the same as in the plasma. Therefore, the glucose levels are elevated in plasma, they are proportionately elevated in the RBCs.

Figure 2B. Higher concentrations of glucose lead to increased formation of HbA1c. Hemoglobin glycation occurs when glucose readily attaches to one, or both, of the N-terminal valines of the \( \beta \)-chain to form a Schiff's base. This fast, unstable process can then slowly form an irreversible keto-amine (Amadori rearrangement). This stable ketoamine form remains for the life span of the RBC.
siderable variation in reference intervals and results reported by different laboratories.

The state of clinical laboratory HbA1c analyses in 1993 was in disorder. The College of American Pathologists (CAP) proficiency surveys for that year demonstrated significant bias and imprecision among the various methods. For example, in 1993, a patient’s HbA1c result of 9% at one hospital could be measured as 16% at another hospital using the same patient sample. The goal of the NGSP was to develop a plan for HbA1c standardization that would allow individual clinical laboratories to relate their assay results to those of large-scale studies such as the DCCT or UKPDS, where the relationship of HbA1c values to mean blood glucose and risks for developing chronic diabetic complications were established. In 1996, the NGSP was initiated to fulfill this goal by standardizing HbA1c test results among laboratories to DCCT-equivalent values. According to the CAP survey results, 99% of those laboratories who reported HbA1c results used a NGSP-certified method by the year 2007, compared to 0% in 1995 (see Figure 3). Additional

A global approach to the standardization of HbA1c was initiated by the International Federation of Clinical Chemistry (IFCC) in 1995.

The NGSP laboratory network currently includes an array of assay methods, each calibrated to an interim reference method (cation-exchange HPLC) that was used in the DCCT to quantify HbA1c. The ADA recommends that all laboratories performing HbA1c measurements participate in the CAP proficiency-testing survey. To help further the standardization process, CAP surveys have used fresh whole-blood specimens with NGSP-assigned target values since 1996. Following the initiation of the NGSP in 1996, the survey has documented a steady improvement in comparability of HbA1c values among laboratories, both within-method and between-method. In general, NGSP-certified methods demonstrate less variability and better comparability to NGSP-assigned target values than other non-certified methods. According to the CAP survey results, 99% of those laboratories who reported HbA1c results used a NGSP-certified method by the year 2007, compared to 0% in 1995 (see Figure 3). Additional

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information regarding the process of NGSP certification and lists of NGSP Certified Methods and Laboratories can be found at www.ngsp.org.

**International standardization efforts**

A global approach to the standardization of HbA1c was initiated by the International Federation of Clinical Chemistry (IFCC) in 1995. While the NGSP was geared toward a national approach to HbA1c standardization, the IFCC established a working group to achieve uniform international HbA1c standardization and to also develop an established reference method to specifically measure glycated Hb for the NGSP to use as a new target standard. Two reference methods were developed to specifically measure the glycated N-terminal residue of the β-chain of Hb (HbA1c). In both of these methods, blood is first washed with saline, after which the RBCs are incubated in NaCl for four hours at 37°C and finally lysed with H2O. Next, the hemolysate is incubated with the endoproteinase Glu-C for 18 hours at 37°C to cleave Hb into peptides, whereby the specific glycated and non-glycated N-terminal peptides of the β-chain of Hb are measured by either capillary electrophoresis or electrospray ionization mass spectrometry. These reference methods were approved by the IFCC in July 2001. Although these reference methods are extremely specific, they are time consuming, technically difficult, lengthy, and very expensive, and, therefore, not suitable for routine clinical-laboratory use.

The IFCC has made significant progress. It developed reference HbA1c-measurement procedures, prepared primary reference material of highly purified HbA1c, developed a reference laboratory network, and performed comparisons with NGSP and other networks. The relationship between the IFCC and NGSP methods, however, using the new IFCC calibration standard demonstrates that IFCC results are consistently ~1.5% to 2% lower throughout the range of HbA1c values. This relationship between the NGSP and IFCC networks has been evaluated; and, as a result, a master equation has recently been developed to document this relationship. This equation (available at www.ngsp.org) can, therefore, be used to link IFCC results to clinically meaningful HbA1c results from the DCCT and the UKPDS. The master equation will also provide the NGSP with traceability to a higher order reference method. Although the IFCC/NGSP correlation is excellent, the absolute numbers are different and the introduction of this calibration standard into clinical laboratories would likely cause great confusion for patients and clinicians. The position of the IFCC Working Group on this subject is to avoid confusion and report the reference method units of mmol of HbA1c/mol Hb instead of derived NGSP units (%) using the IFCC/NGSP master equation.

There has been much debate about which numbers should be reported. The ADA, the European Association for the Study of Diabetes (EASD), and the International Diabetes Foundation (IDF), as well as other member associations in different countries, currently provide patient-care guidelines that relate directly to DCCT/UKPDS numbers. As a result, the ADA/EASD/IDF Working Group of the A1c Assay was established in 2004 to harmonize HbA1c reporting. A consensus statement from the IFCC and the ADA/EASD/IDF Working Group published in October 2007 states that HbA1c test results should be standardized worldwide, including the reference system and results reporting. The Group states that the new IFCC reference system for HbA1c represents the only valid method to implement standardization of the measurement and recommend HbA1c results be reported worldwide in IFCC units (mmol of HbA1c/mol Hb) and derived NGSP units (%), using the IFCC/NGSP master equation. Additionally, an “Average Plasma Glucose Study” is ongoing. If this study fulfills its specified criteria, an HbA1c-derived average glucose (ADAG) value calculated from the HbA1c result will also be reported as an interpretation of the HbA1c. This consensus also states that glycemic goals appearing in all clinical guidelines should be expressed in IFCC units, derived NGSP units, and as ADAG values.

### Pathobiology of HbA1c and Hb variants

Glycation is the non-enzymatic addition of a sugar molecule to amino groups of proteins. Hemoglobin (Hb) glycation occurs when glucose attaches to one or both of the N-terminal valines of the β-chain to form a Schiff’s base. This fast, unstable process can then slowly form an irreversible ketamine (Amadori rearrangement). This stable ketoamine form remains for the life span of the RBC. Glycated hemoglobin (GHb), also sometimes referred to as hemoglobin A1c (HbA1c), glycohemoglobin, HbA1, or A1c, are terms used to describe a series of stable minor Hb components formed slowly and non-enzymatically from Hb and glucose. In order to eliminate the confusing nomenclature regarding GHb and clinical-laboratory testing, the term “A1c test” has been suggested by the National

<table>
<thead>
<tr>
<th>GHb (%)</th>
<th>mg/dL</th>
<th>mmol/L</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>65</td>
<td>3.5</td>
<td>Non-diabetic range</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>135</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>170</td>
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<tr>
<td>8</td>
<td>205</td>
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</tr>
<tr>
<td>12</td>
<td>345</td>
<td>19.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Relationship between HbA1c results and mean plasma glucose. Adapted from the National Glycohemoglobin Standardization Program (available at www.ngsp.org).
Effect of Hb Variants on Ion-Exchange Elution Profile

<table>
<thead>
<tr>
<th>Effect of Hb Variants on Equation Used to Calculate HbA1c</th>
<th>Overall Effect of Hb Variants on HbA1c Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proper separation of HbA from HbA1c</td>
<td>Normal estimation of HbA1c</td>
</tr>
<tr>
<td>Proper separation of both HbA from variant HbX and HbA1c from variant HbX</td>
<td>Normal estimation of HbA1c</td>
</tr>
<tr>
<td>Variant HbX elutes with HbA1c</td>
<td>Extreme overestimation of HbA1c</td>
</tr>
<tr>
<td>Variant HbX1c elutes with HbA1c, and proper separation of HbA from variant HbX</td>
<td>Overestimation of HbA1c</td>
</tr>
<tr>
<td>Proper separation of variant HbX1c from HbA1c, and co-elution of HbA and variant HbX</td>
<td>Underestimation of HbA1c</td>
</tr>
</tbody>
</table>

Table 3. Effects of Hb variants on the calculation of HbA1c using ion-exchange chromatography. Magnitude and direction of expected changes are indicated by arrows next to respective analytes. Hb variants denoted as X represent all possible Hb variants that may result in an abnormal retention time as described in the table. Adapted from References 2, 12, 27.

Academy of Clinical Biochemistry (NACB) guidelines for the laboratory analysis in the diagnosis and management of diabetes mellitus. HbA1c, consisting of HbA1a, HbA1b, and HbA1c, received its name because of its fast-eluting nature as quantified with cation-exchange chromatographic methods. HbA1a and HbA1b comprise only ~20% of HbA1c, while HbA1c, the major component, accounts for the remaining ~80%. Glycation of hemoglobin occurs through mass action whereby the extent of glycation is determined by the hemoglobin concentration and the duration and magnitude of glucose elevation. HbA1c is one of several GHbs that reflect glycemic status, yet it is the form of GHb that has been studied most extensively.

HbA1c values are not only influenced by glucose concentration but also by RBC survival.27 The rate of synthesis of HbA1c is a function of the amount of glucose to which RBCs are exposed over their average 120-day life span (see Figure 2). This relationship serves as a clinically useful index of mean glycemia during the preceding 120 days.26 A normal individual with an average concentration of glucose for the past three months within the normal range (60 mg/dL to 100 mg/dL, 3.5 mmol/L to 5.5 mmol/L) should have a percent HbA1c of <6.5. Several studies have confirmed the findings from the DCCT and have led to the established relationship between HbA1c and mean plasma glucose currently followed by the ADA (see Table 2). In cases where the life span of a patient’s RBCs are altered (shortened or extended) however, diabetes-management guidelines using this established relationship between HbA1c and mean glycemia (during the preceding 120 days) might not be valid. For example, falsely high HbA1c values in relation to a mean blood-glucose values can be obtained when red-cell turnover is low, resulting in a disproportionate number of older red cells. This problem can occur in patients with iron, vitamin B12, or folate deficiency.29 In contrast, rapid red-cell turnover leads to a greater proportion of younger red cells and falsely low HbA1c values. This occurs in patients with hemolysis and those treated for iron, vitamin B12, or folate deficiency, or patients with Hb variants, like HbS or HbC.27 Currently, there are no established guidelines for the use of HbA1c in diabetics with altered RBC survival. In addition, in some cases when the Hb molecule is mutated (Hb variant), some methodologies may demonstrate a significant misrepresentation of HbA1c values. This misrepresentation, however, has proven to be dependent on the methodology used and the particular Hb variant.27

In the United States, this devastating disease accounts for more than $132 billion in healthcare costs annually, and these costs are predicted to rise as high as $192 billion by the year 2020.

Clinical laboratory analysis of HbA1c

There are currently more than 30 commercially available methods to measure HbA1c. The two most common principles include those based on charge differences (ion-exchange chromatography and electrophoresis), and structural differences (boronate-affinity chromatography and immunoassay) of HbA1c.27 According to the 1995 CAP proficiency surveys, 54% of laboratories reporting HbA1c values used affinity chromatography methods. Interestingly, however, CAP surveys from 2007 revealed a sweeping change demonstrating that the majority of laboratories (>97%) had instead begun using immunoassay and ion-exchange methods (see Figure 3), which are more suitable for routine clinical-laboratory use. This progressive change was also likely a result of the increas-
ing use of HbA1c as a measure of average glycemic control, guidelines for the management of diabetes established from the DCCT stressing the importance of the utilization of HbA1c, and the enhanced performance characteristics that exceed classical methods, especially in their non-susceptibility to erroneous HbA1c measurements in the presence of common Hb variants such as HbS and HbC.

According to 2007 CAP proficiency surveys, the ion-exchange chromatography method, in which Hb species are separated based on charge differences, accounted for a little more than 32% of the methods used for the measurement of HbA1c. Typically, these methods used cation-exchange chromatography, in which Hb species elute from the cation-exchange column at different times, depending on their charge, with the application of buffers of increasing ionic strength. The concentration of Hb is measured after elution from the column, which is then used to quantify HbA1c by calculating the area under each peak. Consequently, changes or mutations in the Hb molecule that result in a charge difference from the wild-type Hb species, may or may not alter the normal elution time of those Hb species when using cation-exchange chromatography. Table 3 demonstrates the equation used to determine the amount of HbA1c in a given sample, and the effect of changes in the Hb elution time on HbA1c values when Hb variants cannot be separated from HbA or HbA1c. Although alterations in the ionic strength of mobile phases used in some ion-exchange chromatography methods have reduced the level of co-elution of some of the more common Hb variants (HbS and HbC), it is recommended that clinicians consider methods other than HbA1c for determining average blood-glucose monitoring, such as glycated albumin or fructosamine, given the confusing factors in interpreting HbA1c results for these individuals.

Another method that continues to be used for HbA1c measurement in the clinical lab is boronate-affinity chromatography. This method distinguishes structural differences of GHb species and demonstrates the least interference from the presence of Hb variants and derivatives among commercially available methods. The method can be used to determine total GHb, HbA1c, and ketoamine structures formed on lysines and N-terminal valine residues of both the α- and β-chains of Hb. The principle of this method results in the immobilizing of total GHb to a column that consists of m-aminophenylboronic acid cross-linked to agarose or glass beads. Addition of sorbitol dissociates the complex and elutes all GHb species. Total GHb, as well as HbA1c, may then be measured using spectrophotometry or by quenching of Hb fluorescence. One commercial method has reported no interference from common Hb variants or derivatives. Erroneous measurements in total GHb values, however, have been reported with other commercial methods in HbAC and HbCC variant samples. Boronate affinity accounted for a little more than 2% of the methods used to determine GHb values according to the 2007 CAP proficiency surveys.

Immunoturbidimetric assays accounted for the majority (65%) of the methods used to measure HbA1c according to the 2007 CAP surveys. These methods quantify HbA1c using antibody-mediated inhibition of latex agglutination. Monoclonal and polyclonal antibodies (Abs) used in some of these methods recognize the N-terminal glycated amino acid in the context of the first four
to 10 amino acids of the Hb β-chain. These Abs do not recognize the reversible Schiff base or other GHb species. Unfortunately, the most commonly encountered Hb mutations, HbS (Hb β-chain amino acid 6, Glu > Val) and HbC (Hb β-chain amino acid 6, Glu > Lys), are susceptible to interferences when using primary Abs that recognize amino acid 6 of the Hb β-chain. Other Hbs that have been shown to cause decreased HbA1c values include HbF, HbGraz, and Hb Raleigh. Any variant that results in changes in the first four to 10 N-terminal amino acids of the β-chain of Hb could produce erroneous results when using these Abs. Some methods, however, are not, in fact, affected by common Hb variants. A list of approximately 11 common immunoturbidimetric HbA1c methods and related studies regarding the accuracy of HbA1c measurement in the presence of common Hb variants is available at www.ngsp.org. To reduce the chance of Hb variants causing erroneous HbA1c measurements using immunoturbidimetry, an increasing number of commercial vendors offering these methods now use monoclonal Abs specific for the glycated form of amino acid 2 of the Hb β-chain. Although immunoturbidimetric methods offer the advantage of low cost and automation in the clinical laboratory, an obvious and significant disadvantage of using this method to measure HbA1c is its potential inability to distinguish those patients harboring Hb variants. In contrast to ion-exchange chromatography and boronate-affinity methods, which can sometimes detect Hb variants, the “closed tube” principle of immunoturbidimetric assays does not allow the user to recognize spurious alterations in Hb elution profiles or patterns. For these reasons, immunoturbidimetric method selection should be considered very cautiously in populations where the prevalence of Hb variants is high.

The recommended approach to evaluating or considering the presence of an Hb variant includes a measurement of >15% for HbA1c, or a large change in HbA1c when using a different method. Also, if an Hb variant interferes with the method used by your laboratory, the use of a different assay is suggested. Finally, if the Hb variant alters RBC turnover, consider an alternative for monitoring average blood-glucose control, such as glycated albumin or fructosamine. Regrettably, no large clinical trials have been performed, as with HbA1c, to establish guidelines or goals for the use of glycated albumin or fructosamine values in diabetics in assessing the risks for developing chronic diabetic complications.

In cases where Hb variants preclude the use of a method to determine HbA1c, the fundamental component of HbA1c to manage diabetic patients proves useless. To date, ion-exchange chromatography and immunoturbidimetric methods are most commonly used to measure HbA1c. Although most of these methods used are unaffected by common heterozygous variants, there are currently no established guidelines or goals for clinicians or diabetic patients regarding the use of HbA1c values in these populations. This is especially true for diabetic patients where there is no HbA present, such as homozygous HbS, HbC, or with HbSC disease, as all HbA1c methods are inadequate for the assessment of long-term average blood glucose in these patients due to pathological conditions due to the affect on the formation and turnover of RBCs and the glycation of the N-terminal β-chains of Hb in vivo. This is what had occurred in the patient described in the case study above. The patient suffered from HbC disease in addition to diabetes. The HbC variant resulted in a shortened life span of the patient’s RBCs causing a decrease in the measured HbA1c.

Summary and conclusions

In this article, we have provided a review of the implications of diabetes and HbA1c, the standardization efforts of HbA1c as a long-term monitor of average glycemia, the pathobiology of HbA1c, as well as current measurement pitfalls associated with clinical-laboratory measurements. Long-term studies, including the DCCT and UKPDS, have established a correlation between average blood glucose (HbA1c) and the severity of diabetic complications, thereby providing clinicians and diabetic patients with established HbA1c goals to reduce these problems. This led to an increased need for standardization across all laboratories that perform HbA1c measurements. Through their significant efforts, the NGSP and IFCC have paved the way toward achieving this goal.

Despite these advances, the various HbA1c methods that are currently available each have specific limitations associated with the presence of Hb variants. In areas where there is a high prevalence of Hb variants, HbA1c methods must be carefully selected to reduce the inaccuracy of these measurements. Alternative methods of determining average blood-glucose control (e.g., glycated albumin and fructosamine) are recommended in these populations in which HbA1c determinations have limited value. Unfortunately, as of yet there are no established guidelines or goals that can be followed by clinicians or diabetic patients regarding HbA1c or other glycated protein values in these populations. Importantly, clinicians should be cautious when using glycated albumin and fructosamine as an estimate of long-term average blood glucose. First, glycated albumin and fructosamine assess the degree of glycemia over a period of ~two weeks, as opposed to two to three months, for HbA1c. Second, glycated albumin and fructosamine have not been correlated with the development of long-term complications from diabetes mellitus, as was shown with HbA1c in the DCCT or UKPDS.


