Choosing the Best Method for Hb A\textsubscript{1c} Testing

The measurement of hemoglobin A\textsubscript{1c} (Hb A\textsubscript{1c}, glycated hemoglobin) is an important tool in the diagnosis and management of patients with diabetes. While clinical laboratories make vital contributions to the care of these patients, the method used by those laboratories for assay of Hb A\textsubscript{1c} must attain the required accuracy and precision so these contributions are maximized. There are a few choices when it comes to testing methodology for Hb A\textsubscript{1c} and determining the right method for your practice depends on certain factors. At Grady Memorial Hospital (Grady)—a 953-bed level 1 trauma center in Atlanta—the clinical chemistry laboratory recently confronted a predicament in regard to the accuracy of the method being used for Hb A\textsubscript{1c} measurement.

In addition to containing one of the busiest ERs in the country, Grady offers active programs in surgery, neurology, and burn care, to name a few, as well as an in-demand diabetes clinic within the hospital. To support these programs, the clinical chemistry laboratory is a 24-7 operation, turning out six to seven million test results per year. In general, laboratory-based testing methods are preferred if they are highly automated, efficient, and require minimal interaction from our busy technical staff. Hence, the Hb A\textsubscript{1c} testing method employed for at least the past decade has been an instrument-based automated immunoassay. It was after we adopted a new chemistry instrument that our issue revealed itself.

**Confounding Factors**

The first commercialized methods for Hb A\textsubscript{1c} testing were based on ion exchange chromatography. Through the efforts of the National Glycohemoglobin Standardization Program (NGSP), the clinical value of Hb A\textsubscript{1c} measurement has been enhanced to the point that the quantity is now used to assist in the diagnosis of diabetes as well as monitor the patient’s clinical status. Because Hb A\textsubscript{1c} testing does not require the patient to be fasting and Hb A\textsubscript{1c} concentration is independent of stress, exercise, or other acute factors, it is quickly becoming favored as a diagnostic tool in clinic settings. Of course, for Hb A\textsubscript{1c} to be elevated glucose will have had to be out of control for at least several weeks; thus, Hb A\textsubscript{1c} is not as useful in diagnosing diabetes in acute settings.

While Hb A\textsubscript{1c} clearly has worth as a marker of glucose control, other factors can confound its use and these must be taken into account when interpreting Hb A\textsubscript{1c} results. As with most lab tests, biologic variability influences Hb A\textsubscript{1c}, and evidence exists that patients can exhibit variable glycation rates. Perhaps related to this phenomenon, race seems to influence Hb A\textsubscript{1c}, as Hb A\textsubscript{1c} tends to be higher in African Americans, Asians, and Hispanics than in whites when each person’s glucose control is equivalent by other criteria. Furthermore, any condition altering the RBC life span will vary the amount of Hb A\textsubscript{1c} regardless of the glucose control. For example, patients with hemolytic disease will have shortened RBC survival and will exhibit reduced Hb A\textsubscript{1c} values even when glucose is elevated.

Lastly, a significant number of patients possess Hb variants that arise from mutations in the genes coding for the \(\alpha\) and \(\beta\)-chains. The most common hemoglobin variants in the US are Hb S, C, E, and D, and the prevalence of these variants is dependent on race.

**Immunoassay Approach**

The immunoassay approach to measuring Hb A\textsubscript{1c} is one of the most popular because it is almost fully automated and is included as part of the menu of most large chemistry instruments. In general, Hb A\textsubscript{1c} is determined by two approaches that measure it separately from other (nonglycated) hemoglobins: One based on structural differences and one based on charge differences (see Table 1).

The percentage of Hb A\textsubscript{1c} in a person’s serum/plasma is a valuable indicator of that individual’s glucose control over approximately the previous 120 days, assuming a normal red blood cell (RBC) survival. In fact, the American Diabetes Association (ADA) recommends performing Hb A\textsubscript{1c} testing a minimum of twice a year for patients whose glycemic control is stable, or four times per year for patients who require changes in therapy or have Hb A\textsubscript{1c} values above the clinical goal. Treatment goals arise from studies such as the Diabetes Control and Complications Trial (DCCT), which established a starting goal for patients being treated for diabetes to reach values <7.0% Hb A\textsubscript{1c}.

**Table 1**

<table>
<thead>
<tr>
<th>Analytical Methods for Hb A\textsubscript{1c}</th>
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<tbody>
<tr>
<td>Structural Differences</td>
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<tr>
<td>Affinity chromatography</td>
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<td>Immunoassay</td>
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**Historical Note**

The measurement of Hb A\textsubscript{1c} was first described by two teams in 1965—Embree and co-workers, and Kise and colleagues. The Embree group initially utilized liquid chromatography coupled with a fluorometric detector to measure Hb A\textsubscript{1c}, while Kise’s group employed an immunoassay method. Since then, Hb A\textsubscript{1c} has become a widely used tool for diabetes diagnosis and management. Today, laboratories perform Hb A\textsubscript{1c} testing in various ways, including chromatography, electrophoresis, immunoassay, and mass spectrometry. Each method has its strengths and weaknesses, and the choice of method depends on factors such as cost, turnaround time, and patient population. For instance, chromatographic methods are considered the gold standard for accuracy, but they are also the most expensive and time-consuming. On the other hand, immunoassays are faster and less expensive but may not be as accurate as chromatographic methods for all patient groups. Mass spectrometry is a newer technology that offers high accuracy and specificity but is still in the research phase and not widely available. Therefore, laboratories must carefully consider the trade-offs when selecting an Hb A\textsubscript{1c} testing method to ensure they are providing the best care for their patients.
Roughly 8–12% of African Americans have the Hb S trait, while 3% of people of African descent have the Hb C trait. Hb traits affect Hb A1c interpretation in two ways: People with these traits have altered RBC life spans (although current data is mediocre) and analytical interference occurs with some methods. This second point is critical when selecting the best method for your practice.

**Finding the Right Fit**

As mentioned, there are two main approaches to determining Hb A1c (structural and charge based), which have led to the hundred or more commercially available methods available today. Because of the large number of alternatives, the decision faced by laboratory managers is challenging. However, the selection of the right method for your practice should be made using the same process that has been successful for years: Know the analytical requirements for precision and accuracy needed for the analyte, identify the features and specifications needed from the device, and find the most cost-effective method that best meets established requirements. Several recent guidelines have set the following analytical goals for Hb A1c:

- **Precision** should be ≤ 2% CV or ≤ 0.3% Hb A1c
- **Accuracy** should be within 6% of the true value (i.e., if the true value is 5.5% Hb A1c, the method bias should be ≤ 0.33% Hb A1c)

These may seem like stringent goals, especially for a potential point-of-care (POC) application, but they are necessary to maximize the integrity of the test.

Therefore, it is essential to understand that the accuracy of the Hb A1c method you select may be most influenced by the population of patients you serve. At Grady, we serve a significant African American and Hispanic population, so selecting an Hb A1c method plagued by interference from an Hb S and/or C trait would be a poor choice. When we discovered we were having accuracy issues with Hb A1c, we realized it was due to having switched from one major chemistry instrument to another. The methods employed by both the old and new instruments were immunoassay based, but with most newer instruments, the immunoassays used are at least second generation where the capture antibody is directed against the first four amino acids. The antibody for earlier (first generation) immunoassays was directed against amino acids 4–10. The mutation for the S and C hemoglobin chains occurs at position 6; thus, the earlier assays could not accurately determine Hb A1c in human carriers of the Hb S or C traits.

Surprisingly (and unfortunately), the method used by the new instrument was found to be a first generation immunoassay, but we were unaware of this fact during the method evaluation phase prior to purchase. It was not until some months into the use of the new instrument that we discovered this problem, as our clinicians began contacting us about several patients that appeared to have excellent glucose control, but who had been resulted with Hb A1c values indicative of poor control. The confusion was cleared when it was discovered that all the patients in question carried either the Hb S or C trait, and we were able to confirm with our instrument’s vendor that the Hb A1c testing method had remained first generation.

**Lab-based and POC**

Discovering this unfortunate situation after the fact led us to seek out an alternate, supplemental method; one that obviously would avoid interference from patients likely to have the S or C traits, but also would still meet our requirement of offering substantial freedom from operator interaction. Fortunately, there are a number of instruments now available that meet this necessity, as even HPLC and other separation methods, such as affinity chromatography and electrophoresis, are available on highly automated instruments. We evaluated three different lab-centric instruments and selected one based on affinity chromatography, which showed a lack of interference from the common hemoglobin variants, high throughput, rapid time to first result, and significant automation, as well as met our analytical quality requirements.

Shortly after implementing this instrument, several of our neighborhood-based clinics began requesting on-site Hb A1c capability. However, the instrument we chose for our main laboratory possessed several features that were not required in the POC setting, namely, high capacity. At the two clinics that made the initial request, the expected volume was only about 4–10 samples per day, but we still required the same analytical quality as expected in the main laboratory. After reviewing the available POC instruments we felt would achieve the accuracy we required, we selected one based on the same boronate affinity chromatography approach used in the main lab. This was done in part to help standardize Hb A1c results across the Grady Health System. Fortunately, most Hb A1c methods available today have gained certification by the NGSP and are thus traceable for accuracy compared to the DCT, but if multiple methods are required in the same institution, care should be taken to select only methods that have been certified by the NGSP. The NGSP website (www.ngsp.org/interf.asp) also contains information on whether methods exhibit interference from Hb traits.

**Conclusion**

With all of the acceptable methods available for Hb A1c testing today, most laboratories can offer the test themselves—either through POC or lab-based instrumentation—assuming there is a volume of at least five per day (any less than that may dictate sending this test out to a reference laboratory). With this in mind, perhaps the most important information to gather when determining the best method by which to facilitate Hb A1c at your facility is to consider the populations served and whether your instrumentation can negotiate common hemoglobin variants.

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