Accuracy and Clinical Utility of a Point-of-Care HbA$_{1c}$ Testing Device

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Abstract

Background: Point-of-care testing (POCT) is widely used to measure blood glucose levels in people with diabetes, although its use in measuring glycated hemoglobin (HbA$_{1c}$) levels is less common, perhaps due to perceived performance issues and access to the technology. Methods: Forty blood samples were analyzed in duplicate using Bayer’s A1CNow® Multi-Test A1C system (A1CNow+) with 3 different reagent lots; HbA$_{1c}$ levels of the samples spanned the clinically relevant range of 4% to 10%. Corresponding samples were sent to a National Glycohemoglobin Standardization Program (NGSP) secondary reference laboratory (University of Missouri Secondary Reference Laboratory #9), which analyzed the samples with a Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh G8; Tosoh Bioscience, Inc). Results: Glycated hemoglobin levels measured with the A1CNow+ aligned with measurements obtained using the laboratory method, with correlation coefficients of 0.985, 0.987, and 0.989 for the 3 lots, respectively. The 95% CIs for the differences between the A1CNow+ levels and the mean HbA$_{1c}$ levels were within −0.55% to +0.50% for the 3 reagent lots, which is well within the currently acceptable limits of ±0.75% HbA$_{1c}$ required by the NGSP. Results were further analyzed per the new tighter NGSP performance criteria effective September 1, 2012, requiring that 37 of 40 results be within ±7% (relative bias) of the NGSP reference laboratory measures. All 3 lots met the tighter NGSP criteria. Conclusion: The A1CNow+ provides accuracy and precision when performing POCT of HbA$_{1c}$, as an aid in diabetes management. Ongoing improvements in this and other HbA$_{1c}$ POCT devices may lead to a greater global acceptance of the role of POCT of HbA$_{1c}$ in diabetes management.

Keywords: point-of-care testing; glycated hemoglobin; diabetes management; HbA$_{1c}$ measuring devices

Introduction

The incidence of diabetes in the United States is approaching 8% of the population and is on the rise around the world. Since the publication of the Diabetes Control and Complication Trial (DCCT) results in 1993, and subsequently the United Kingdom Prospective Diabetes Study (UKPDS), it has been acknowledged that improved control of blood glucose is the key parameter to forestalling serious complications of the disease. New tools and technology may help to achieve this end. One such tool is point-of-care testing (POCT) of glycated hemoglobin (HbA$_{1c}$) levels, which allows the patient, together with a health care provider, to discuss the patient’s glycemic status in a single, face-to-face encounter, which can encourage more vigorous glucose control measures that may lead to better outcomes. However, the clinical utility of POCT depends on the availability of accurate and convenient HbA$_{1c}$ test systems that are affordable and easy to use in the clinician’s office.
**HbA\textsubscript{1c} POCT**

Glycated hemoglobin is a sensitive predictor of the long-term complications of diabetes. High HbA\textsubscript{1c} levels are strongly linked to increased risk of cardiovascular disease, nephropathy, and retinopathy,\textsuperscript{7,8} and predict most of the excess mortality risk in men with diabetes.\textsuperscript{9} The Centers for Disease Control and Prevention state that “every percentage point drop in HbA\textsubscript{1c} blood test results (eg, from 8.0% to 7.0%) can reduce the risk of microvascular complications (ie, eye, kidney, and nerve diseases) by 40%.”\textsuperscript{11} At the present time, the American Diabetes Association (ADA) and the American Association of Clinical Endocrinologists recommend that, in general, most adults with diabetes strive for an HbA\textsubscript{1c} level < 7% and < 6.5%, respectively.\textsuperscript{10,11}

For patients with diabetes, as well as for those with many other diseases, POCT allows a timely response to a clinical question, and therefore may offer advantages over a delayed response that may occur when using laboratory-based tests.\textsuperscript{12} Point-of-care testing can reduce the time between health care provider/patient contact and the initiation, modification, or termination of therapy. In addition, laboratory analysis may allow opportunity for patient noncompliance with the instruction to obtain the test, a known obstacle to the effective management of both type 1 and type 2 diabetes mellitus.\textsuperscript{13,14} Individuals who are advised to have blood drawn for the laboratory test may fail to do so, or may fail to attend the required follow-up visit. Point-of-care testing, in contrast, is a patient-centered approach that allows monitoring of prognosis and compliance with the therapeutic regimen in a single visit, obviating the need for the patient to return for the results. In addition, POCT has the potential to reach a wider range of people than conventional laboratory testing, particularly in regions where conventional laboratory testing is inaccessible or otherwise unavailable. The demand for POCT is increasing in many areas, leading to improving technologies and guidelines.\textsuperscript{12,15–18} Nonetheless, it has engendered some controversy, largely based on concerns about accuracy and precision.\textsuperscript{10,19–21}

The National Glycohemoglobin Standardization Program (NGSP) has made tremendous strides in the standardization of HbA\textsubscript{1c} testing and has established guidelines and protocols for evaluating all HbA\textsubscript{1c} measurement instruments, whether for laboratory testing or POCT.\textsuperscript{22} To be certified by the NGSP, an HbA\textsubscript{1c} testing method must meet standards for performance in order to demonstrate traceability to the DCCT and UKPDS study methods used to measure HbA\textsubscript{1c} levels. Thus, the HbA\textsubscript{1c} measurement results produced in clinical practice by any NGSP-certified method can be more confidently compared with that of any other NGSP-certified method. Specifically, a manufacturer must perform a sample comparison with a Secondary Reference Laboratory (SRL) within the NGSP network using samples that cover a specified range of HbA\textsubscript{1c} levels (4%–10%). In 2011, the criteria for NGSP manufacturer certification required that the result be within 0.75% HbA\textsubscript{1c} (tightened from 0.85% in 2010) of the SRL level, with 95% confidence.\textsuperscript{23} Beginning in September 2012, the NGSP criteria for certification were substantially tightened. These criteria apply to methods annually certified after September 1, 2012, and will thus apply to all certified methods by September 1, 2013. Changes in NGSP criteria generally require a 1-year rollover period for currently certified methods. The new performance requirements for manufacturers and level II laboratories are that 37 of 40 results be within ±7% (relative bias) of the SRL measurements. The 40 blood samples will be analyzed in singleton and the results will be compared with the mean of duplicate SRL measurements.\textsuperscript{24} Thus, based on these requirements, an HbA\textsubscript{1c} test result measured in the 4% to 10% HbA\textsubscript{1c} level range can be up to 7% (relative bias) higher or lower than the true (reference) result. This means that an HbA\textsubscript{1c} level measured as 7.0% could indicate a true HbA\textsubscript{1c} level anywhere in the range of 6.51% to 7.49%. In comparison, the previous NGSP performance requirements allowed for an HbA\textsubscript{1c} test result measured as 7.0% to indicate a true level anywhere in the range of 6.25% to 7.75%.

In addition to NGSP standardization, the International Federation of Clinical Chemistry (IFCC) has also developed reference methods (eg, mass spectroscopy and capillary electrophoresis) for HbA\textsubscript{1c} analysis and has established a laboratory network. Although there is excellent correlation between IFCC and NGSP measurements, IFCC measurements are 1.5% to 2% HbA\textsubscript{1c} lower over the spectrum of HbA\textsubscript{1c} levels. The relationship between the NGSP calibration,\textsuperscript{25} which is expressed as percentage of HbA\textsubscript{1c} and the IFCC network calibration, which is expressed as mmol/mol, has been shown to be stable and is obtained by the master equation: NGSP = (0.09148 x IFCC) + 2.152.

Beyond the need for standardization, an HbA\textsubscript{1c} POCT device should also be easy to use by untrained users. In the United States, the US Food and Drug Administration (FDA) is charged with verifying whether use of a clinical test is sufficiently straightforward, such that specific types of regulatory oversight of its use can be waived. Formally, a waiver from the Clinical Laboratory Improvement Amendments (CLIA) can be granted for a simple
A limitation of the current technology is that, while hemoglobin E and hemoglobin D do not interfere with the A1CNow+, sickle cell hemoglobin and hemoglobin C do interfere with this system, which may report inaccurate (falsely high) results when used in patients with these types of hemoglobin variants.27

Methods
A study was conducted to determine the analytical performance of the A1CNow+. Forty specimens of de-identified, heparin-preserved, fresh whole blood from internal and commercial sources were analyzed at the Bayer facility (Sunnyvale, CA) using the A1CNow+. The entire analysis was performed 3 times, each time with a different reagent lot. Samples were treated in the same manner as would they be used by an end user, as were all associated reagents, calibration materials, and software. The HbA1c levels of the samples spanned the clinically relevant range of 4% to 10%, with 20% of the levels between 4% and 5.5%, and 20% between 8.5% and 10%. The remainder were divided equally between 5.5% and 7%, and 7% and 8.5%. The testing based on each of the 3 lots occurred over a period of 5 days and each specimen was analyzed in duplicate. Corresponding samples were sent to an NGSP SRL (University of Missouri SRL #9). The NGSP laboratory analyzed the samples with a Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh G8; Tosoh Bioscience, Inc). Bayer personnel were blinded to the HbA1c test results from the NGSP SRL until completion of the data analysis.

Results
The HbA1c levels obtained with A1CNow+ were plotted against the corresponding levels obtained using the laboratory method (Figure 1). The calculated correlation coefficients (r) were 0.985, 0.987, and 0.989 for reagent lots 1, 2, and 3, respectively (Table 1).

The difference between the A1CNow+ measurements and the laboratory measurements is plotted against the mean HbA1c level in Figure 2. The upper and lower confidence limits of the 95% CI for the 3 lots were +0.45% and −0.55%; +0.50% and −0.48%; and +0.32% and −0.52%, respectively. All of these levels were well within the NGSP acceptable limits of ±0.75% HbA1c (Table 1). The coefficients of variation for the 3 datasets were 3.2%, 2.1%, and 2.9% based on analysis of the duplicate results (Table 1). An analysis was also performed to determine if the 3 lots would pass the new tighter NGSP criteria effective September 1, 2012; all 3 lots met the tighter NGSP criteria (Table 2; Figure 3).
Figure 1. Scatter plot showing A1CNow® HbA₁c measurements compared with those obtained via the NGSP Secondary Reference Laboratory method for A) lot 1, B) lot 2, and C) lot 3. Each sample was assayed in duplicate with each of the 3 reagent lots.

Abbreviations: HbA₁c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program.
### Table 1. A1CNow® Performance Evaluated by NGSP Criteria Effective Through August 31, 2012

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Lot 1</th>
<th>Lot 2</th>
<th>Lot 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation (r)</td>
<td>0.985</td>
<td>0.987</td>
<td>0.989</td>
</tr>
<tr>
<td>CV, %</td>
<td>3.2</td>
<td>2.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Upper CL of the 95% CI, %</td>
<td>+0.45</td>
<td>+0.50</td>
<td>+0.32</td>
</tr>
<tr>
<td>Lower CL of the 95% CI, %</td>
<td>−0.55</td>
<td>−0.48</td>
<td>−0.52</td>
</tr>
<tr>
<td>NGSP acceptable limit of ±0.75% HbA₁c</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Abbreviations:** CV, coefficient of variation; CL, confidence limit; HbA₁c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program.

### Discussion

The measurement of HbA₁c level has become a recognized component in the management of diabetes. However, notwithstanding the demonstrated value of POCT in the management of diabetes, POCT of HbA₁c is not used in clinical practice as frequently as conventional laboratory testing. Both the ADA and the International Diabetes Federation endorse the acquisition of an HbA₁c test result at the time of a patient’s visit with his or her health care provider.¹⁰,²⁸

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**Figure 2.** Assessment of agreement between the A1CNow® HbA₁c measurements and those obtained via the NGSP Secondary Reference Laboratory plotted against the mean of the 2 measurements for **A** lot 1, **B** lot 2, and **C** lot 3 based on NGSP criteria effective through August 31, 2012.

**Abbreviations:** HbA₁c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program.
Table 2. A1CNow® Performance Evaluated by NGSP Criteria Effective September 1, 2012

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Lot 1: R1</th>
<th>Lot 1: R2</th>
<th>Lot 2: R1</th>
<th>Lot 2: R2</th>
<th>Lot 3: R1</th>
<th>Lot 3: R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c levels within ±7% of measurements obtained via the SRL</td>
<td>37/40</td>
<td>40/40</td>
<td>39/40</td>
<td>38/40</td>
<td>40/40</td>
<td>37/40</td>
</tr>
<tr>
<td>NGSP acceptable limit of 37 of 40 results</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Abbreviations: HbA1c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program; R, replicate; SRL, Secondary Reference Laboratory.

Figure 3. Assessment of agreement between the A1CNow® HbA1c measurements and those obtained via the NGSP Secondary Reference Laboratory plotted against the mean of the 2 measurements for A) lot 1, B) lot 2, and C) lot 3 based on NGSP criteria effective September 1, 2012.

Abbreviations: HbA1c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program.
These circumstances suggest that POCT devices, such as the A1CNow®, may play an increasingly important role in the management of diabetes in the future.

The application of POCT to HbA$_{1c}$ measurements may afford the benefits of rapid reporting of clinical test results, timely adjustments to therapeutic regimens, fewer missed laboratory appointments, and potentially reduced administrative time and costs. A challenge to future researchers examining the performance of HbA$_{1c}$ POCT devices would be to include analysis of all of the relevant information, including laboratory device variability, in order to provide a broader context for interpretation.

Laboratory proficiency surveys conducted by the College of American Pathologists indicate that in the field, variation within and between laboratory-based methods can be comparable with or sometimes greater than some POCT results. Including such considerations would shed light on realistic performance expectations for current HbA$_{1c}$ testing methods. Although performance should be of primary concern, additional clinical considerations such as patient access, cost, portability, convenience, and the impact of immediately available HbA$_{1c}$ test results would also add value to the context of this discussion.

**Conclusion**

The A1CNow® has been improved since its introduction, such that it has continued to receive yearly certification despite ever-tightening NGSP standards. The latest version of the device has incorporated multiple modifications to its manufacturing, calibration, and optics that collectively support its current analytical performance. Moreover, it is a CLIA-waived, IFCC-traceable, handheld device that has been found to be accurate and convenient to use in several reports. Ongoing improvements in the A1CNow® and other HbA$_{1c}$ POCT devices, such as the in2it, DCA Vantage, and Afinion, may contribute to wider acceptance of HbA$_{1c}$ POCT for monitoring HbA$_{1c}$ levels, and promote the availability of effective tools for management of glycemic control in people with diabetes.

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**Conflict of Interest Statement**

Jennifer Knaebel, RN, MSN, Benjamin R. Irvin, PhD, and Charles Z. Xie, MD, are full-time employees of Bayer HealthCare LLC, Diabetes Care.

**References**