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# Hemoglobin A1c: Assessment of three POC analyzers relative to a central laboratory method

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ABSTRACT

*Background:* Glycosylated hemoglobin evaluation is very important for assessing the control of diabetes. Since the use of point-of-care (POC) devices for monitoring HbA1c is increasing, it is important to determine how these devices compare in relation to instrumentation used in the central laboratory (CL).
*Methods:* Eighty-eight randomly selected samples previously analyzed using the Bio-Rad Variant™ II Hemoglobin Testing System were run on three POC Analyzers (Siemens DCA Vantage™ Analyzer, Axis-Shield Afinion™ AS100 Analyzer, and Bio-Rad In2it™ Analyzer).
*Results:* All POC instruments showed good correlation to the CL method ($R^2$>0.95 for all methods). HbA1c levels obtained using Variant II (mean = 7.9; 95% CI = 7.5–8.3%) and In2it (mean = 7.9; 95% C.I. = 7.5–8.2%) instruments were found to have no statistical mean difference ($p = 0.21$), while the values obtained using DCA Vantage (mean = 7.2% C.I. = 6.9–7.5%) and Afinion (mean = 7.3% C.I. = 7.0–7.6%) instruments were different ($p<0.001$) from those of the CL method. The Afinion and DCA Vantage instruments increasingly underestimated the HbA1c compared to the CL as the HbA1c values increased. These differences were even more striking when the estimated average glucose is calculated.
*Conclusions:* Despite significant variation of results among the POC instruments evaluated relative to the CL method and pending resolution of HbA1c standardization issues, we conclude that all of the POC instruments can be used for HbA1c determination if clinicians are given instrument specific reference ranges.


## 1. Introduction

The use of hemoglobin A1c (HbA1c), also known as glycated hemoglobin or glycosylated hemoglobin, in the management of diabetes is well established [1–4]. It is currently one of the most important markers for the long-term assessment of glycemic status and monitoring the effect of therapy in patients with diabetes. This has been shown by 2 landmark outcome studies, the Diabetes Control and Complication Trial (DCCT) [3] and the United Kingdom Prospective Diabetes Study (UKPDS) [4]. Although HbA1c testing has historically been performed in central laboratories, it has been shown that patients can be managed as well or possibly better when HbA1c is measured at point-of-care (POC) [5–8].

Recently the American Diabetes Association (ADA) has recommended that the HbA1c be monitored using an optimal HbA1c target of <7%. For some patients, the ADA has indicated that HbA1c should be as close to normal (<6%) as possible without episodes of significant hypoglycemia [2]. The ADA recommendations concerning glycemic targets also emphasize the need for accurate and precise instrumentation in monitoring serial HbA1c measurements in individual patients. Recently it was shown that an analytic bias exists for the most commonly used POC HbA1c method relative to a central laboratory (CL) method [9]. In addition the same authors identified similar though lesser biases for various clinical laboratory HbA1c methods. With the increased use of POC HbA1c analyzers and given that such methods are not typically used for high-volume testing in CL settings, a patient might be serially tested using multiple HbA1c methods (POC and CL) within the same healthcare system. It is thus imperative that differences in results be known and communicated to physicians. To investigate these potential differences, we compared the HbA1c values obtained using three different POC analyzers (DCA Vantage, Afinion and In2it) to those of the Variant II method used in the CL.

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## 2. Materials and methods

### 2.1. Instruments and reagents

We compared the HbA1c results determined by POC analyzers and our CL analyzer using whole blood specimens submitted to the CL for the purpose of patient care between March and June 2008. These samples were analyzed using the following POC analyzers: Axis-Shield (Abbott Diagnostics, Abbott Park, IL) Afinion™ AS100 Analyzer, Bio-Rad In2it™ Analyzer (Hercules, CA), and Siemens DCA Vantage™ Analyzer (Tarrytown, NY). The Bio-Rad Variant II system (CL method) result was used as the reference method to which all of the POC analyzers were compared. The Bio-Rad Variant II system is a cation-exchange HPLC that is NGSP and IFCC certified. The imprecision of the system was 4.4% and 2.6% at levels of 5.7% and 9.5% HbA1c, respectively. Testing by all methods was completed within 24 h after sample arrival in the clinical laboratory. All methods have recently been certified in 2010 by the National Gylcohemoglobin Standardization Program (NGSP) (http://www.ngsp.org/prog/index.html): DCA Vantage in June 2010, Afinion in September 2009, In2it in August 2009, and Variant II in January, 2010. Briefly, the DCA Vantage Analyzer measures HbA1c by immunoassay (immunoglutination inhibition) in a small (1 μL) sample of EDTA whole blood in 6 min. The Afinion and In2it analyzers use affinity chromatography (boronic acid) to separate glycated and nonglycated hemoglobin. The Afinion Analyzer uses 1.5 μL of EDTA whole blood to determine the HbA1c result in 3 min, and the In2it Analyzer uses 5 μL of EDTA whole blood to determine the HbA1c result in 10 min.

### 2.2. Specimens

Institutional review board approval was obtained for the use of 88 specimens designated for discard that had been submitted to the CL for the purpose of routine patient care from inpatient and outpatient clinics. HbA1c was tested on whole blood specimens collected in tubes containing EDTA as the anticoagulant. The values (obtained using the CL method) ranged between approximately 5% and 12%.

### 2.3. Imprecision

Since hemolysis interferes with the analysis of HbA1c on the Afinion, pooled patient samples were not used to evaluate imprecision of the POC analyzers. Instead imprecision at 2 levels for the In2it and DCA Vantage analyzers was assessed using DCA 2000 HbA1c controls. Because the DCA 2000 HbA1c controls gave error codes for the Afinion Analyzer imprecision was assessed using the Afinion HbA1c controls. Within-run imprecision was determined by 10 replicate analyses of each of 2 levels of control. Between-run imprecision was also determined before and after analysis of patient samples for 2 levels of controls daily over 8 days.

### 2.4. Statistical analysis

All results were expressed as mean ± SD. The results were analyzed using SAS v 9.2 (Cary, NC, USA) to determine the significance between the groups. Paired t-tests were used to determine significance of the differences between the means based on the methodology of measuring HbA1c. To access correct $p$-values a permutation (randomization) test using a re-sampling without replacement was also performed. This has the advantage of making no distributional assumptions (such as normality, equal variance) about the data. A 2-tailed $p<0.05$ was considered the criterion for statistical significance. Passing and Bablok regression (MedCalc v 11.3.3.0, Mariakerke, Belgium) with 95% CI was used for regression analysis and graphical representation of the comparison data.

## 3. Results

We investigated three POC analyzers (Afinion, DCA Vantage, and In2it) for use in our outpatient clinics. As part of this study, we compared the POC analyzers with the CL method (Variant II) by identifying 88 samples from patients sent to the CL for routine HbA1c analysis. The HbA1c values for these samples ranged from 5.2% to 11.4%. Two of the samples gave an error code with the Afinion Analyzer, indicating a level of hemolysis that would interfere with the analysis. No error codes were seen with the DCA Vantage or the In2it analyzers. While hemolysis should not be a problem in most patients, in some patients a difficult finger stick can cause hemolysis in the sample. The correlation of the various POC analyzers and the Variant II method is shown in Table 1 and was found to be excellent for the Afinion, DCA Vantage, and In2it ($R^2=0.977$, 0.973, 0.951, respectively), with corresponding regression equations of $y=0.83*\text{Variant}+0.70$, $y=0.81*\text{Variant}+0.78$, $y=0.85*\text{Variant}+1.10$, respectively (see Fig. 1a–c for graphical representation). The levels of HbA1c obtained using the In2it Analyzer were found to have no statistical mean difference when compared to the results obtained using the Variant II method (mean of Variant II = 7.9%; mean of In2it = 7.9%; $p=0.21$). This was confirmed using a permutation test which is a method that randomizes the results to evaluate how the actual structure of data compares to random rearrangements of data ($p=0.414$). However, HbA1c values obtained using Afinion and DCA Vantage analyzers were significantly different from those of the Variant II method (mean of Afinion = 7.3% and mean of DCA Vantage = 7.2%, $p<0.001$). Below an HbA1c of 6% (upper limit of normal), all analyzers, POC and CL, give very similar results, with means of 5.7%, 5.5%, 5.4%, and 5.9% for Variant II, Afinion, DCA Vantage,

**Table 1**
Analysis of results for split samples using Variant II and POC HbA1c methods.

| Linear regression analysis | Variant | Afinion | DCA Vantage | In2it |
|---|---|---|---|---|
| X = central laboratory | | | | |
| Y = POC | | | | |
| N | 88 | 86 | 88 | 88 |
| Slope (SE) | | 0.83 (0.014) | 0.81 (0.014) | 0.85 (0.021) |
| Intercept (SE) | | 0.70 (0.113) | 0.78 (0.117) | 1.10 (0.169) |
| $R^2$ | | 0.977 | 0.973 | 0.951 |
| Mean (95% CI) | 7.9% (7.5–8.3%) | 7.3% (7.0–7.6%) | 7.2% (6.9–7.5%) | 7.9% (7.5–8.2%) |
| Difference (95% CI) | | 0.65% (0.57–0.74%) | 0.76% (0.67–0.85%) | 0.06% (−0.03–0.15%) |
| Significant deviation from linearity ($p$-value) | | <0.05 | <0.05 | 0.21 |
| Permutation test ($p$-value) | | 0.004 | 0.0013 | 0.414 |