Evaluation of the Afinion AS100 Point-of-Care Analyzer for Hemoglobin A_{1c}

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Abstract: An evaluation of a new Afinion AS100 Analyzer was conducted to assess analytical performance. Precision was estimated by analyzing 2 control and 3 patient samples twice a day for 10 days. Accuracy was established by analysis of 6 samples from the National Glycated Hemoglobin Standardization Program for a 3-day period. Agreement was correlated to a laboratory method, the Variant II Turbo Hemoglobin Testing System, and a point-of-care method, the DCA2000+ Hemoglobin A_{1c} System, using leftover EDTA samples from laboratory analysis (n = 110, range of results = 4.6%–13.7% HbA_{1c}). The Afinion AS100 Analyzer (0.9%–1.8% coefficient of variation [CV]) displayed laboratory comparable precision (Variant II Turbo = 1.1%–1.9% CV) that was superior to the DCA2000+ (2.9%–3.3% CV) with minimal bias to the National Glycated Hemoglobin Standardization Program target concentrations (<0.2% HbA_{1c} average unit bias or 3.1%). The Afinion AS100 Analyzer had good agreement with both the Variant II Turbo and DCA2000+ with *r* of greater than 0.9837 and Sy.x of ±0.22% and ±0.29% HbA_{1c}, respectively. Staff found the analyzer easy to train and use, providing faster results than the DCA2000+ (3 minutes vs 6 minutes). The Afinion AS100 Analyzer will be recommended over the DCA2000+ when requests for future point-of-care HbA_{1c} are made in our health system.

Key Words: hemoglobin A_{1c}, glycated hemoglobin, point-of-care

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Glycated hemoglobin is produced by the nonenzymatic attachment of glucose to the amino acid groups of hemoglobin (Hb). Adult human Hb consists of 97% HbA with the remainder HbA_2 and HbF. Chromatographic analysis of HbA identifies several minor "fast" Hbs that migrate more rapidly than HbA in an electric field, namely HbA_{1a}, HbA_{1b}, and HbA_{1c}, collectively referred to as HbA_1, glycohemoglobins, glycosylated Hbs, or more properly termed glycated Hb.[1] Blood levels of glycated Hb A1c (HbA_{1c}) depend on the average blood glucose concentration and can be used as a marker of diabetic control over the previous 2 to 3 months, given the 120-day life span of the red blood cell and Hb molecules.

Diabetes is the sixth leading cause of death in the United States.[2,3] There are 20.8 million children and adults in the United States or approximately 7% of the population with diabetes. Diabetes accounts for nearly half the new cases of renal failure and 12,000 to 24,000 cases of blindness annually. Costs of diabetic complications totaled more than $132 billion in 2002.[4] The Diabetes Control and Complications Trial (DCCT) linked glucose control with reduction in long-term risk of diabetes complications including retinopathy, neuropathy, and nephropathy.[5] The DCCT study identified glycated Hb levels as a primary risk factor for development of diabetes complications,[5] and for every 1% reduction in HbA_{1c} levels, the risk of developing eye, kidney, and nerve disease is reduced by 40%.[2,3]

Monitoring HbA1c levels as a means of lowering average blood glucose levels is therefore recommended by several professional organizations including the American Diabetes Association (ADA),[6] the American Association of Clinical Endocrinologists,[7] the Centers for Disease Control,[8] and the Veterans Affairs Administration.[9,10] The Health Plan Employer Data and Information Set[3] recommends monitoring HbA_{1c} at least annually, whereas the ADA[6] and American Association of Clinical Endocrinologists[7] advocate for measuring HbA_{1c} at every follow-up visit or once every 3 months. The ADA has established target goals for glycated Hb based on the DCCT trial of less than 7% HbA_{1c} and for individuals to aim as close to normal (<6% HbA_{1c}) as possible without significant hypoglycemic episodes.[6]

There are a variety of methods for performing glycated Hb analysis including electrophoresis, immunoassay, high-pressure liquid chromatography (HPLC), affinity, and ion-exchange chromatography.[1] Method and calibration differences can lead to variation among results from different methodologies. Given the goals set by professional societies for glycated Hb levels independent of analytical methodology, a National Glycated Hemoglobin Standardization Program (NGSP) has established certification of analytical methods with traceability to the DCCT study and standardization of method calibration.[11] The NGSP primary reference laboratories use an HPLC Bio-Rex 70 resin protocol with fresh blood samples to establish set points for primary calibrators, controls, and other materials used to administer the NGSP.

Most glycated Hb methods are performed in a central laboratory. This requires patients to obtain a physician order, have their blood drawn, and wait for results to be sent back to their physician for interpretation and any changes in clinical management. Several point-of-care methods are now available that are certified by the NGSP and allow for analysis of HbA_{1c} in the physician's office. There is a growing body of evidence that testing for HbA_{1c} in the physician's office leads to faster patient treatment and improved outcomes, including enhanced physician and patient satisfaction. The National Academy of Clinical Biochemistry has developed practice guidelines recommending the use of point-of-care HbA_{1c} in the primary and secondary health care setting.[12,13] The major benefit of point-of-care HbA_{1c} is from the diabetic specialist having the result at the time of patient counseling. The HbA_{1c} availability in the physician's office streamlines patient management by providing testing in the office at the time the patient is being examined. This ensures compliance with Health Plan Employer Data and Information Set, ADA, and other HbA_{1c} frequency of monitoring recommendations because physicians

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TABLE 1. Precision Study Results

Control	n	Mean (%)	SD (%)	CV (%)
Afinion AS100 Analyzer				
Control C I	40	6.1	0.06	0.9
Control C II	40	7.8	0.08	1.1
Low Patient	40	5.5	0.10	1.8
Mid Patient	40	6.3	0.09	1.3
High Patient	40	8.0	0.14	1.7
DCA2000+				
Control 1	4	5.2	0.17	3.3
Control 2	4	10.3	0.30	2.9
Variant II Turbo (A)				
Control 1	32	5.8	0.08	1.3
Control 2	33	9.8	0.11	1.1
Variant II Turbo (B)				
Control 1	35	5.7	0.11	1.9
Control 2	35	9.6	0.11	1.1

n indicates number of replicates.

do not have to send patients for a separate phlebotomy and risk loss to follow-up.

This study was conducted to evaluate the analytical performance of a new point-of-care HbA_{1c} method, the Afinion AS100 Analyzer (Axis-Shield PoC Norton, Mass), and to assess the analyzer's potential for future use in the Baystate Health System. The Afinion AS100 Analyzer was compared with our current methods, the DCA2000+ Hemoglobin A_{1c} System (abbreviated as the DCA2000+; Siemens Medical Solutions Diagnostics, Tarrytown, NY) and the Variant II Turbo Hemoglobin Testing System (abbreviated as the Variant II Turbo; Bio-Rad Laboratories, Hercules, Calif), for assay precesion, accuracy, and method correlation. Operational features and ease of use were also assessed during the evaluation.

MATERIALS AND METHODS

The study was conducted at Baystate Medical Center in Springfield, Mass, by a medical laboratory technologist and pathology resident after training and documentation of competency on both the Afinion AS100 Analyzer and the DCA2000+. The Afinion AS100 Analyzer and test cartridges were provided for this evaluation by the manufacturer, Axis-Shield PoC. All other supplies were obtained from stock in production for clinical testing. The DCA2000+ and supplies were purchased from Siemens Medical Solutions Diagnostics, and the Bio-Rad Variant II Turbo and supplies were purchased from Bio-Rad Laboratories. One Afinion AS100 analyzer, 1 DCA 2000+, and 2 Bio-Rad Variant II Turbos were used for this study.

Precision for the Afinion AS100 Analyzer was evaluated by analyzing 3 patient and 2 control samples, in duplicate, twice a day, for 10 days. The protocol for this study was reviewed by our institutional review board and found to be exempt from review. Specimens were leftover samples from laboratory analysis with patient identifiers removed. Specimens were collected in EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and analyzed as soon as possible after completion of clinical testing (within the same 8-hour shift). Specimens were mixed well by rocking on a rotating platform at least 15 minutes before analysis. For the precision study, patient samples were stored refrigerated (2°C–8°C) and warmed to room temperature before analysis.

Accuracy was evaluated by analysis of 6 samples from the NGSP for a 3-day period. Samples were stored refrigerated (2°C–8°C) and warmed to room temperature before analysis by each method. For the Variant II Turbo, the NGSP samples were analyzed in singlicate on each of the 2 Hemoglobin Testing Systems, labeled as Variant II Turbo (A) and Variant II Turbo (B), to evaluate for any bias between analyzers.

Method correlation was conducted by analyzing fresh patient specimens in singlicate by each method. Specimens were analyzed by all methods within the same 8-hour shift. The presence of hemoglobinopathies was documented when detected by the Variant II Turbo, and the HbA_{1c} results were reviewed for possible interference or abnormal bias outside of the assay precision. All specimens were performed on the DCA 2000+ and Afinion AS100 Analyzer but were randomized between the 2 Variant II Turbos according to the routine laboratory workload. Correlations were completed over a 1-week period.

Data reduction was performed using Statistica 5.1 software package for Microsoft Windows (Stat-Soft, Tulsa, Okla). Analytical precision was estimated from control and patient replicates on the Afinion AS100 Analyzer. Precision for comparative methods was estimated from analysis of manufacturer recommended controls over the same time frame. For accuracy, the total unit and percent bias was calculated from the NGSP target concentrations for each standard, and the reproducibility of each NGSP standard was estimated to evaluate for possible sample matrix interferences. Method correlation and correlation statistics were estimated by least squares regression. Statistical difference for mean bias and method agreement were evaluated by the student *t* test.

RESULTS

Precision on the Afinion AS100 Analyzer varied from 0.9% to 1.1% coefficient of variation (CV) over the range of 6.1% to 7.8% HbA_{1c} for control samples and 1.3% to 1.8% CV

TABLE 2. Accuracy Study Results

	Average Unit Bias (Range)	Percent Bias (Range)	Reproducibility
Afinion AS100 Analyzer	−0.23% HbA_{1c} (−0.5 to 0.0)	−3.1% (−6.5 to 0.0)	0.8%–2.9% CV
DCA2000+	−0.38% HbA_{1c} (−1.2 to 0.0)	−4.9% (−10.7 to 0.0)	0.0%–4.6% CV
Variant II Turbo (A)	0.05% HbA_{1c} (−0.4 to 0.3)	1.1% (−3.6 to 5.5)	0.9%–1.8% CV
Variant II Turbo (B)	−0.05% HbA_{1c} (−0.3 to 0.1)	−0.5% (−2.7 to 1.8)	0.0%–1.0% CV

Average unit bias and percent bias to target concentration of 6 NGSP standards. Range of results and reproducibility of values are also shown. Study conducted for 3 days, n = 3 replicates per NGSP standard.

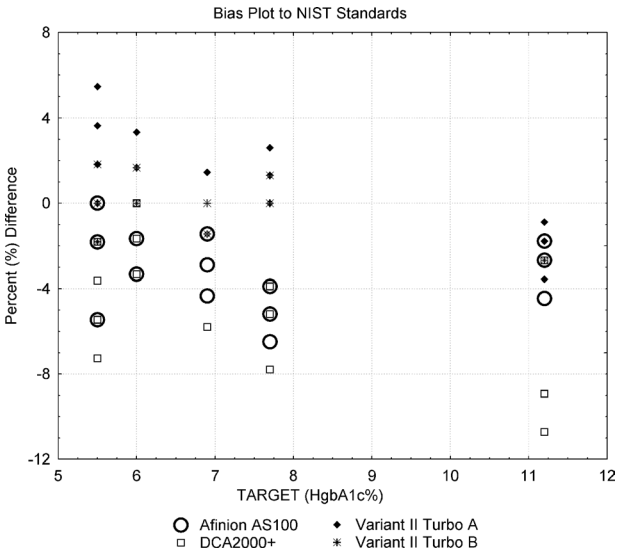

FIGURE 1. Bias plot of percent differences (*y* axis) for the Afinion AS100 Analyzer, DCA2000+ and 2 Variant II Turbo systems compared with the target HbA_{1c} concentrations for NGSP standards (*x* axis). Note that 2 of the NGSP standards had the same target concentration of 5.5% HbA_{1c}.

over the range of 5.5% to 8.0% HbA_{1c} for patient samples (Table 1). This compares with the Variant II Turbo precision of 1.1% to 1.9% CV over the range of 5.8% to 9.8% HbA_{1c} for control samples and is better than the DCA2000+ with precision of 2.9% to 3.3% CV over the range of 5.2% to 10.3% HbA_{1c} for control samples.

Bias was assessed by comparison to target concentrations of 6 NGSP standards analyzed over 3 days. The average unit and percent bias was calculated for each method (Table 2). The DCA2000+ had the lowest bias (mean bias = −0.38% HbA_{1c} or −4.9%; statistically significant difference from NGSP target at the $P < 0.001$ level), whereas the Variant II Turbos were closest to the NGSP target concentrations (Variant II Turbo [A] mean bias = 0.05% HbA_{1c} or 1.1%; Variant II Turbo [B] mean bias = −0.05% HbA_{1c} or −0.5%, not statistically significant at the $P = 0.05$ and $P = 0.001$ levels). The Afinion AS100 Analyzer demonstrated low bias that was intermediary to the Variant II Turbo and DCA2000+ methods with a mean bias of −0.23% HbA_{1c} or −3.1% (Afinion AS100 Analyzer was statistically different from NGSP target concentrations at the $P < 0.001$ level). Whereas the Afinion AS100 Analyzer bias was consistent throughout the range of the NGSP standards, both the DCA2000+ and the Variant II Turbo displayed higher bias at lower HbA_{1c} values and lower bias in the higher range of HbA_{1c} values (Fig. 1). Reproducibility of the NGSP standards ranged from 0.0%–1.8% CV for the Variant II Turbo, which is consistent with the precision seen on control samples. However, both the Afinion AS100 Analyzer (0.8%–2.9% CV) and DCA2000+ (0.0%–4.6% CV) had higher variability on some of the NGSP standards that could indicate a possible matrix effect or interference with these methods from the NGSP standards that were not demonstrated with fresh patient specimens.

A total of 110 patient specimens were analyzed for method correlation. The samples ranged from 4.6% to 13.7% HbA_{1c}. Five samples were identified by the Variant II Turbo to have Hemoglobin S, and 1 sample had Hemoglobin C, but the presence of these hemoglobinopathies did not affect the HbA_{1c} analysis, so these samples were included in the correlations. All samples were analyzed by the Afinion AS100 Analyzer and DCA2000+, but the samples were split between the 2 Variant II Turbo systems based on the timing of routine clinical workload in the laboratory (n = 65 for Variant II Turbo [A] and n = 45 for Variant II Turbo [B]). Scatterplots demonstrate good correlation between the Afinion AS100 Analyzer and comparative methods with correlation coefficients (*r*) of greater than 0.9837. The (Sy.x) of the estimates for the Afinion AS100 Analyzer was ±0.22% HbA_{1c} compared with the Variant II Turbo and ±0.29% HbA_{1c} compared with the DCA2000+. The correlation study demonstrated similar bias to the accuracy study, with the Variant II Turbo generating higher results (mean of patient results = 7.9% HbA_{1c}) than the DCA2000+ (mean of patient results = 7.4% HbA_{1c}). The Afinion AS100 Analyzer results were statistically different at the $P < 0.001$ level by the Student *t* test and were intermediary between the

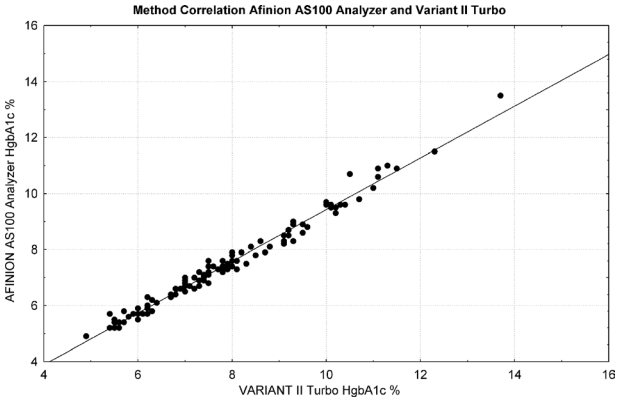

FIGURE 2. Method correlation Afinion AS100 Analyzer compared with the Variant II Turbo HbA_{1c} concentration. Least squares regression equation, Afinion AS100 Analyzer (y) = 0.194 + 0.924 Variant II Turbo (x); correlation coefficient *r* = 0.9899; SE of the estimate, Sy.x = 0.22% $HgbA_{1c}$ for patient specimens (N = 110).

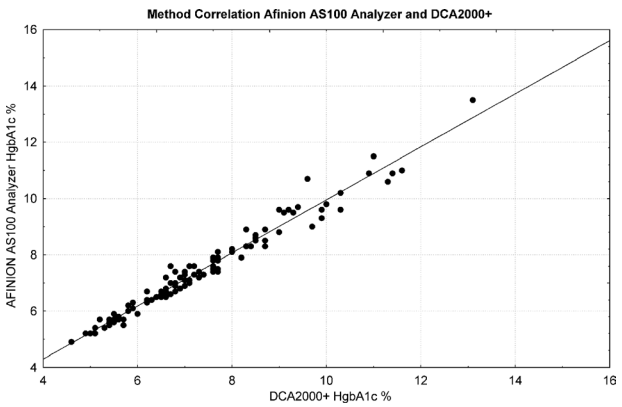

FIGURE 3. Method correlation Afinion AS100 Analyzer compared with the DCA2000+ HbA_{1c} concentration. Least squares regression equation, Afinion AS100 Analyzer (y) = 0.53 + 0.943 DCA2000+ (x); correlation coefficient *r* = 0.9837; SE of the estimate, Sy.x = 0.29% $HgbA_{1c}$ for patient specimens (N = 110).

Variant II Turbo and DCA2000+ (Afinion AS100 Analyzer mean of patient results = 7.5% HbA_{1c}) (Figs. 2, 3).

DISCUSSION

The Afinion AS100 Analyzer performed remarkably well. Precision was superior to the DCA2000+ and comparable to a laboratory HPLC method, the Variant II Turbo. The Afinion AS100 Analyzer had minimum bias to the NGSP standardization program and was closer to target values than the DCA2000+. The Afinion AS100 Analyzer correlated with both the DCA2000+ and Variant II Turbo methods. Although the Afinion AS100 Analyzer and DCA2000+ results were statistically different than our laboratory Variant II Turbo and target NGSP concentrations, these differences were not clinically significant. Without a consensus on HbA_{1c} agreement, the College of American Pathologists proficiency surveys can be used to judge analytical performance that would impact clinical significance. The College of American Pathologists has set target ranges for HbA_{1c} analytical performance within ±3 SD of the analyzer peer group.[14] For the DCA2000+, 1 SD is between 2% to 5% CV, for a total acceptable range of ±6% to 15% of the DCA2000+ peer group. By contrast, the Swedish government has set HbA_{1c} analytical goals of less than 3% CV or a total acceptable range of ±9%.[15] Both the Afinion AS100 Analyzer and DCA2000+ met these goals for analytical performance overall, although the DCA2000+ demonstrated precision on some levels of controls slightly greater than 3% CV (ie, 3.3% at 5.2% HbA_{1c}). There is further evidence that this performance is clinically acceptable because our physicians are currently using the DCA2000+ in a pediatric endocrinology clinic without issue. The Afinion AS100 Analyzer demonstrated better accuracy and generated results closer to the Variant II Turbo and NGSP target concentrations than our current DCA 2000+, so the Afinion AS100 Analyzer performance should be acceptable to our physicians.

The operators found the Afinion AS100 Analyzer easy to learn and use. The DCA2000+ and Afinion AS100 Analyzer have similar footprints on the bench and are comparable in environmental requirements like operating temperature, humidity, power, and need for a level bench that is stable during operation. Neither the DCA2000+ nor the Afinion AS100 Analyzer is intended to be portable. These devices are more amenable to a physician's office or satellite laboratory setting with dedicated space rather than moved to the patient's bedside, particularly because both analyzers weigh 11 lb. However, this is where the similarities end. The Afinion AS100 Analyzer is faster (3 minutes to result versus 6 minutes for the DCA2000+), stores more data (up to 500 patient and 500 control results), and is barcode compatible for scanning specimen and reagent labels. Staff had very positive comments regarding the ease of use, particularly sample loading during analysis.

Although the results of this study were very positive, there were some study limitations to consider. The DCA2000+ is only used by our laboratory to analyze specimens with hemoglobinopathies that could interfere with our Variant II Turbo method, so the number of controls performed on this analyzer is less than the other methods examined in this study. A lower number of control replicates for the DCA2000+ could bias the precision results. However, our precision for the DCA2000+ were comparable to results reported by other studies.[16,17] The analytical range of controls was narrower for the Afinion AS100 Analyzer that could also have contributed to better precision with this method. Another potential limitation of this study was the inclusion of 5 samples with hemoglobinopathies in the correlation. The presence of altered Hbs is typically detected by the Variant II Turbo chromatograms but must be known before analysis with other methods (although all methods tested have extensive interference validations from common hemoglobinopathies during market approval). These hemoglobinopathy specimens were included in the correlations for 2 reasons: (1) no HbA_{1c} result was more than 3 SD or 9% (assuming a 3% CV) different from the other methods, indicating a potential outlier; and (2) in routine outpatient practice, the physician would not be alerted to the potential for interference from hemoglobinopathies before testing with the device. Even with inclusion of these 5 samples, there was good agreement and a high degree of correlation between the methods. Finally, the use of patient samples from a single week in our core laboratory may not be reflective of analyzer performance in all of our outpatient settings. One potential criticism is that the study was conducted in a laboratory environment by medical technologists and pathology residents who are more skilled than staff in the outpatient clinics. Differences in operator use and patient populations could give different performance characteristics in a clinic compared with a laboratory setting. However, samples from these same clinics are part of our routine clinical workload and were included in this evaluation. So the potential for possible differences was controlled as best as possible by including clinic specimens in our evaluation. Operator factors could not be assessed in the laboratory, however, short of testing the device in a variety of locations, which was not the intent of this evaluation.

In summary, the Afinion AS100 Analyzer was accurate and precise and compared well with our laboratory Variant II Turbo and current point-of-care DCA2000+ methods. The Afinion AS100 Analyzer was easy to use, and staff noted advantages over the DCA2000+, particularly faster analysis time and easier sample loading. Our point-of-care testing committee was impressed with the Afinion AS100 Analyzer performance and will be recommending this analyzer when requests for future point-of-care HbA_{1c} are made to the point-of-care testing committee.

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