Three of 7 Hemoglobin A$_{1c}$ Point-of-Care Instruments Do Not Meet Generally Accepted Analytical Performance Criteria

Erna Lenters-Westra$^{1,2,*}$ and Robbert J. Slingerland$^{1,2}$

BACKGROUND: In 2009, we investigated the conformance of 8 hemoglobin A$_{1c}$ (Hb A$_{1c}$) point-of-care (POC) instruments. Since then, instruments have improved and new devices are available on the market. In this second study, we evaluated the performance of DCA Vantage, Afinion, InnovaStar, Quo-Lab, Quo-Test, Cobas B101, and B-analyst Hb A$_{1c}$ POC instruments.

METHODS: Clinical and Laboratory Standards Institute protocols EP-5 and EP-9 were applied to investigate imprecision, accuracy, and bias. We assessed bias using the mean of 3 certified secondary reference measurement procedures (SRMPs). Assay conformance with the National Glycohemoglobin Standardization Program (NGSP) certification criteria was also evaluated. Interference of common Hb variants was investigated for methods that could work with hemolysed material.

RESULTS: The total CVs for all instruments, except for the DCA Vantage at a high Hb A$_{1c}$ value, were $\leq$3.1% in SI units and $\leq$2.1% in Diabetes Control and Complications Trial (DCCT) units. Afinion, DCA Vantage, B-analyst, and Cobas B101 instruments passed the NGSP criteria with 2 different reagent lot numbers. Quo-Test, Quo-Lab, and InnovaStar instruments had a negative bias compared to the mean of the 3 SRMPs and failed NGSP criteria. Most of the common Hb variants did not interfere with the investigated instruments, except Hb AE for the Cobas B101.

CONCLUSIONS: Afinion, DCA Vantage, Cobas B101, and B-analyst instruments met the generally accepted performance criteria for Hb A$_{1c}$. Quo-Test, Quo-Lab, and InnovaStar met the criteria for precision but not for bias. Proficiency testing should be mandated for users of Hb A$_{1c}$ POC assays to ensure quality.

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Point-of-care (POC)$^3$ is the fastest growing market in clinical chemistry. Today, there are some developments to transition diagnostics from the second line (in the hospital) to the first line (in the family physician’s office) or even to the zero line (in the patient’s home) (1). Ensuring the quality of the POC testing instrument used is crucial and should be embedded in a chain of quality control from hospital to doctors’ offices; otherwise the impact on patients will be immense, especially if these methods will be used for the diagnosis of different diseases.

In 2009, we evaluated 8 different glycated hemoglobin (Hb A$_{1c}$) POC instruments and came to the conclusion that, at that time, 6 of 8 Hb A$_{1c}$ POC instruments did not meet the generally accepted analytical performance criteria of a total CV $<3.0\%$ and a National Glycohemoglobin Standardization Program (NGSP) manufacturer certification (2–4). Since then, some manufacturers have either improved their methods or withdrawn from the market. New instruments have since come to market. This initiated a second round of evaluations of different POC instruments. We approached all manufacturers that joined the first evaluation study and asked them if they were willing to join this second round. Some were still improving their methods and not ready for this second test, and some did not want to join this second round for unknown reasons. In the meantime, manufacturers from new POC instruments approached us and asked us to do an evaluation of their methods, and eventually we had 7 Hb A$_{1c}$ POC instruments to evaluate.

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$^3$ Nonstandard abbreviations: POC, point-of-care; Hb A$_{1c}$, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program; SRMP, secondary reference measurement procedure; MDP, medical decision point; DCCT, Diabetes Control and Complications Trial; RCV, reference change value.
The aim of this study was to evaluate the 7 POC instruments according to the CLSI protocols and to check whether the instruments would pass the NGSP criteria with 2 different reagent lot numbers compared with 3 certified IFCC and NGSP secondary reference measurement procedures (SRMPs). Additionally, we investigated the presence of potential interference from common Hb variants on the instruments capable of analyzing frozen material, and we tried to find out whether the investigated Hb A\textsubscript{1c} POC instruments can be used for the diagnosis of diabetes.

**Materials and Methods**

The 7 POC Hb A\textsubscript{1c} analyzers evaluated in this study were

- The DCA Vantage\textsuperscript{TM} (Siemens Medical Solutions Diagnostics), which is based on latex agglutination inhibition immunoassay methodology and provides results in 6 min.
- The B-analyst (Menarini Diagnostics), which is based on latex agglutination immunology turbidimetric methodology, with results available in 8 min.
- The Afinion\textsuperscript{TM} (Alere Technologies), which is based on boronate affinity separation, with results available in 5 min.
- The Quo-Test (Quotient Diagnostics, an EKF Diagnostics Holding Company), which is based on boronate affinity separation and the use of fluorescence quenching, with results available in 3 min.
- The Quo-Lab (Quotient Diagnostics), which is based on boronate affinity separation and the use of fluorescence quenching, with results available in 3 min (this method is the same as the Quo-Test but needs some manual handling).
- The InnovaStar (DiaSys Diagnostics), which is based on agglutination immunoassay and provides results in 11 min.
- The Cobas B101 (Roche Diagnostics), which is based on latex agglutination inhibition immunoassay methodology and provides results in 5 min.

DCA Vantage, Afinion, Quo-Test, and InnovaStar analyzers were also evaluated in the previous study (2). B-analyst, Quo-Lab, and Cobas B101 are new on the market.

The Menarini B-analyst was evaluated at the end of 2012, and the other 6 instruments were evaluated in January 2014. All instruments were NGSP certified at the time of the evaluation (5). B-analyst and the Afinion can work only with fresh patient material and not with hemolysed or lyophilized material.

To get an overall impression of performance before starting the CLSI EP-5 and EP-9 protocols, IFCC monitoring program samples were analyzed (frozen whole blood samples, n = 24; 12 samples in duplicate) on instruments specified for hemolysed material (6). For those not specified for hemolysed material, 12 fresh patient samples were run in duplicate. The familiarization study was not done with the B-analyst. The values were assigned to the IFCC monitoring samples by the IFCC network laboratories by use of the IFCC primary reference methods (n = 15) (7). Values were assigned to the fresh patient samples by the 3 SRMPs mentioned below. Precision was also calculated by analyzing the samples in duplicate. The results were sent to the manufacturers for their approval to continue with the evaluation. After obtaining manufacturer approval, we used the CLSI EP-5 protocol to further investigate assay imprecision (duplicate measurements twice per day on 2 patient samples for 20 days) (8). Aliquots were made from the patient samples and stored at minus 80 °C degrees until analysis. Controls supplied by the manufacturer were used for Afinion and B-analyst, as these methods are not specified for use with hemolysed material. Because B-analyst was evaluated 1 year earlier, we used a slightly different protocol for the imprecision study. Aside from the controls supplied by the manufacturer of the B-analyst (medium and high value), we also used a fresh sample and analyzed it twice per day in duplicate for 10 days instead of 20 days. Studies in the past have shown that better CVs are achieved with the Afinion when controls are used in an EP-5 protocol rather than patient samples (2, 4). Therefore CVs were also calculated on the basis of the duplicates of the fresh patient samples in the EP-9 protocol for all instruments.

The CLSI EP-9 protocol was performed twice with 2 different reagent lot numbers, and the data were used to investigate the bias between the POC instruments and the 3 SRMPs (n = 40, 5 days, duplicate measurements) (9). Hb A\textsubscript{1c} value determination of the patient samples was performed with 3 certified SRMPs:

- Roche Tina-quant Gen.2 Hb A\textsubscript{1c} on Integra 800, immunoassay, IFCC and NGSP certified (Roche Diagnostics);
- Premier Hb9210, affinity chromatography HPLC, IFCC and NGSP certified (Trinity Biotech); and
- Tosoh G8, cation-exchange HPLC, IFCC certified (Tosoh Bioscience).

The SRMPs have documented good results in the IFCC and NGSP monitoring programs and were calibrated by use of the IFCC secondary reference material with assigned IFCC and derived NGSP values (6, 10, 11). To check overall calibration and bias independently of the chosen SRMP, the results of the POC instruments in the EP-9 procedure were compared with the mean of the 3 SRMPs, and medical decision point (MDP) analysis was performed at an Hb A\textsubscript{1c}
value of 48 mmol/mol [6.5% Diabetes Control and Complications Trial (DCCT) units]. When the 2 methods are statistically identical, the 95% CI for each y MDP includes the corresponding x MDP. The student 2-tailed t-test for paired samples was used to check for statistically significant difference between the 2 lot numbers. A P value <0.05 was considered significant.

The EP-9 protocol was used to evaluate the methods against 3 different SRMPs with 2 reagent lot numbers, and the data were also used to calculate the NGSP certification criteria. Beginning in January 2014, 37 of 40 results need to be within 6% (relative) of an individual NGSP SRMP to pass certification (12).

Interference from common Hb variants Hb AS, Hb AC, Hb AD, Hb AE, increased A2 (β-thalassemia), and Hb F was investigated by the instruments specified to analyze frozen, hemolysed material. Five samples of each variant with different Hb A1c values were analyzed in 1 day. The specific variants were identified/confirmed with cation-exchange HPLC (Menarini HA8180V, Diabetes Mode) and capillary electrophoresis (Sebia Capillars 2 Flex Piercing). Percentage Hb F was determined with the Sebia Capillars 2 Flex Piercing. Hb A1c values were assigned by use of IFCC-calibrated cation exchange HPLC (Premier Hb9210) and, for samples with increased Hb F, Hb A1c values were assigned by use of IFCC-calibrated boronate affinity HPLC (Premier Hb9210) and, for samples with increased Hb F, Hb A1c values were assigned by use of IFCC-calibrated cation exchange HPLC (Menarini HA8180V, Diabetes Mode). A mean relative difference of >6% was considered a significant interference. The results were corrected for bias found in nonvariant samples when calculating the mean relative difference.

STATISTICS

Calculations were performed by use of Microsoft® Excel 2010 (Microsoft Corp.). Statistical analyses were performed by use of Analyse-It® (Analyse-It Software) and EP Evaluator Release 9 (Data Innovations) (13).

For the duplicates in the EP-9 protocol, CV was calculated with the following formula:

\[
CV_{a} = \frac{\sqrt{\sum_{i=1}^{n}(\Delta_i)^2}}{\bar{x}} \times 100\% \tag{1}
\]

where \(CV_{a}\) is the analytical CV and \(\Delta_i\) is the difference between duplicates, \(n\) is the number of duplicates, and \(\bar{x}\) is the mean of the duplicates.

The reference change value (RCV), which is the critical difference in the change in a patient’s serial test results that can be considered significantly different at a CI of 95%, was calculated with the following formula:

\[
RCV (\%) = \sqrt{2} \times 1.96 \times \sqrt{\frac{(CV_a)^2 + (CV_{wp})^2}{n}} \tag{2}
\]

where \(CV_{a}\) is the analytical CV and \(CV_{wp}\) is the intra-individual or biological (within-person) CV (\(CV_{wp}\)) (14, 15).

Results

On the basis of the good results obtained in the familiarization study (deviation from the target value ≤1.9 mmol/mol, reproducibility ≤2.6% in SI units for all instruments), all manufacturers agreed to participate in the full evaluation.

Table 1 shows the results of the EP-5 protocol and the CVs on the basis of the duplicate samples analyzed in EP-9. Precision ranged from 1.3% in SI units at an Hb A1c value of 71 mmol/mol (0.9% at Hb A1c value of 8.7% in DCCT units) for InnovaStar to 4.2% in SI units at an Hb A1c value of 73 mmol/mol (3.2% at Hb A1c value of 8.8% in DCCT units) for DCA Vantage.

Table 2 shows NGSP certification pass/fail criteria with respect to the results of the EP-9 protocol performed with fresh patient samples. The instruments were compared to 3 SRMPs with 2 different reagent lot numbers and also with the mean of 3 SRMPs (Fig. 1, A–D, and Fig. 2, A–C). B-analyst, DCA Vantage, and Afinion passed the NGSP criteria with 2 reagent lot numbers compared with the SRMPs. Cobas B101 passed the NGSP criteria with 2 lot numbers compared with the Tosoh G8 but not when compared with the Premier Hb9210 or the Roche Tina-quant Gen.2 on Integra 800. Bias for Afinion, Cobas B101, B-analyst, and the DCA Vantage was <1.0 mmol/mol compared with the mean of 3 SRMPs with 2 different reagent lot numbers. Quo-Test, Quo-Lab, and InnovaStar showed a significant bias compared with the mean of the 3 SRMPs and failed the NGSP criteria compared with the 3 SRMPs with both reagent lot numbers.

Statistically, we observed no difference between the 2 lot numbers for all instruments. The P values were: Afinion, 0.88; Cobas B101, 0.83; DCA Vantage, 0.69; B-analyst, 0.88; InnovaStar, 0.83; Quo-Lab, 0.88; and Quo-Test, 0.97.

To determine if the evaluated POC devices are capable of diagnosing diabetes, MDP analysis was performed at an Hb A1c of 48 mmol/mol (6.5% DCCT) compared with the mean of the 3 SRMPs with 2 reagent lot numbers (Table 3). No statistical difference was measured between the mean of the 3 SRMPs and B-analyst, Cobas B101, and Afinion for either reagent lot. For DCA Vantage, no statistical difference was observed only for the second reagent lot.

Five frozen samples with different Hb A1c values and different Hb variants [Hb AS, Hb AC, Hb AD, Hb AE, increased A2 (β-thalassemia), and Hb F] were analyzed to investigate for the presence of potential interference. If the results of the investigated Hb variant fall
within the deviation of the nonvariant samples distributed around the regression line, the investigated Hb variant can be considered as “not interfering.” Visual analysis of the graphs (see Supplemental Data for Table 4, which accompanies the online version of this article at http://www.clinchem.org/content/vol60/issue8) shows that all instruments had a negative bias at Hb F 6.9%. The negative bias with Hb F is directly proportional in magnitude to the percentage Hb F present in the sample. The Hb F used in this study ranged from 3.2% to 18.3%. Table 4 shows the mean relative difference of the Hb variants (n = 5 per variant) compared to the assigned value. Most of the instruments do not have interference from Hb AS, Hb AC, Hb AD, Hb AE, or increased A2, except for Cobas B101, which had interference from Hb AE (mean relative difference 17.1% increase). The mean relative difference of Hb AC for the DCA Vantage was 6.9% increase.

**Discussion**

The results of this study showed that the analytical performance of POC instruments has improved considerably compared with the results of the first evaluation study in 2009 (2). In the familiarization study, all instruments showed excellent/good reproducibility in the clinically relevant range (SI units 20–86 mmol/mol, DCCT units 4.0%–10.0%) and minimal bias with frozen material. Unfortunately, the study revealed a major problem regarding calibration/standardization of 3 instruments. The results from the familiarization study were very promising for all manufacturers, and all manufacturers gave approval to continue the evaluation study. The bias found with fresh samples in the EP-9 for Quo-Test, Quo-Lab, and InnovaStar was not expected (Fig. 2, A–C). Additional experiments showed that the same samples, fresh and frozen, gave different results (see online Supplemental Data for Fig. 2, A–C). In daily life, only fresh whole blood (capillary or venous) is used when measuring Hb A1c with a POC instrument. This bias could affect the patients whose Hb A1c is analyzed by these methods by yielding a falsely low result that could possibly affect therapeutic options, which must be based on the true Hb A1c value. This, in turn, could lead to the development of complications that would have been avoided if the instrument were performing acceptably (16, 17). This shows the importance of joining external quality assessment for POC users. The fact that most POC users do not participate in external assessment schemes does not allow manufacturers the opportunity to investigate and potentially fix such problems. In the future, fresh whole blood will be required for calibration, NGSP certification, and IFCC monitoring program for Quo-Test, Quo-Lab, and InnovaStar.

The mean relative difference of 6.9% for Hb AC for the DCA Vantage exceeds the criterion of >6%. However, this criterion is very strict, especially if the Hb A1c values of the Hb AC samples are low. Looking at the graph, only 2 Hb AC samples exceed the 6% criterion and the other samples are within the 6% criterion, which makes it implausible that there is a real interference of Hb AC (see online Supplemental Data for Table 4). More samples with Hb AC and
different Hb A1c values need to be analyzed to confirm our findings.

The precision of the DCA Vantage in the first study in 2009 was 1.8% in DCCT units at an Hb A1c of 5.1% and 3.7% at an Hb A1c value of 11.2%. The CV in the high area was considered high but not clinically relevant. In the current study, we decided to take a lower Hb A1c for the high sample (73 mmol/mol, 8.8% DCCT). The CV again was too high (4.2% in SI units and 3.2% in DCCT units) compared with the general accepted performance criteria (intralaboratory CV <2% in DCCT units, <3% in SI units, and a NGSP manufacturer certification) (12, 18). The question can be raised, what does this mean for daily practice? To calculate the RCV, the CVw is needed, and also the within-person biological variation for Hb A1c (CVwp).

### Table 2. EP-9 method comparison results (Deming regression lines) in DCCT units and pass/fail NGSP certification criteria.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Lot number 1</th>
<th>Bias</th>
<th>SEE</th>
<th>Samples &gt;6% of SRMP, n</th>
<th>NGSP criteria</th>
<th>Lot number 2</th>
<th>Bias</th>
<th>SEE</th>
<th>Samples &gt;6% of SRMP, n</th>
<th>NGSP criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-analyst (y)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>vs Premier (x)</td>
<td>y = 1.08x - 0.41</td>
<td>0.19</td>
<td>0.15</td>
<td>1</td>
<td>Pass</td>
<td>y = 1.08x - 0.41</td>
<td>0.16</td>
<td>0.16</td>
<td>2</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.08x - 0.39</td>
<td>0.19</td>
<td>0.18</td>
<td>0</td>
<td>Pass</td>
<td>y = 1.08x - 0.39</td>
<td>0.16</td>
<td>0.18</td>
<td>1</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 1.08x - 0.46</td>
<td>0.15</td>
<td>0.15</td>
<td>0</td>
<td>Pass</td>
<td>y = 1.08x - 0.46</td>
<td>0.11</td>
<td>0.15</td>
<td>2</td>
<td>Pass</td>
</tr>
<tr>
<td>DCA Vantage (y)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>vs Premier (x)</td>
<td>y = 0.99x - 0.10</td>
<td>-0.15</td>
<td>0.21</td>
<td>3</td>
<td>Pass</td>
<td>y = 1.01x - 0.15</td>
<td>-0.05</td>
<td>0.20</td>
<td>0</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.04x - 0.35</td>
<td>-0.04</td>
<td>0.19</td>
<td>1</td>
<td>Pass</td>
<td>y = 1.06x - 0.39</td>
<td>0.06</td>
<td>0.20</td>
<td>0</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 1.02x - 0.19</td>
<td>-0.06</td>
<td>0.20</td>
<td>0</td>
<td>Pass</td>
<td>y = 1.04x - 0.22</td>
<td>0.03</td>
<td>0.22</td>
<td>1</td>
<td>Pass</td>
</tr>
<tr>
<td>Afinion (y)</td>
<td></td>
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<tr>
<td>vs Premier (x)</td>
<td>y = 0.97x + 0.13</td>
<td>-0.08</td>
<td>0.18</td>
<td>0</td>
<td>Pass</td>
<td>y = 0.91x + 0.60</td>
<td>-0.06</td>
<td>0.15</td>
<td>0</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.02x - 0.08</td>
<td>0.02</td>
<td>0.20</td>
<td>0</td>
<td>Pass</td>
<td>y = 0.95x + 0.39</td>
<td>0.05</td>
<td>0.16</td>
<td>2</td>
<td>Pass</td>
</tr>
<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 0.99x + 0.08</td>
<td>0.00</td>
<td>0.22</td>
<td>2</td>
<td>Pass</td>
<td>y = 0.93x + 0.52</td>
<td>0.03</td>
<td>0.17</td>
<td>3</td>
<td>Pass</td>
</tr>
<tr>
<td>Quo-Test (y)</td>
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<tr>
<td>vs Premier (x)</td>
<td>y = 1.00x - 0.29</td>
<td>-0.30</td>
<td>0.19</td>
<td>7</td>
<td>Fail</td>
<td>y = 0.99x - 0.24</td>
<td>-0.32</td>
<td>0.16</td>
<td>11</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.05x - 0.52</td>
<td>-0.20</td>
<td>0.20</td>
<td>6</td>
<td>Fail</td>
<td>y = 1.04x - 0.47</td>
<td>-0.21</td>
<td>0.18</td>
<td>5</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 1.02x - 0.37</td>
<td>-0.22</td>
<td>0.20</td>
<td>5</td>
<td>Fail</td>
<td>y = 1.01x - 0.32</td>
<td>-0.23</td>
<td>0.18</td>
<td>5</td>
<td>Fail</td>
</tr>
<tr>
<td>Quo-Lab (y)</td>
<td></td>
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<tr>
<td>vs Premier (x)</td>
<td>y = 0.98x - 0.20</td>
<td>-0.35</td>
<td>0.20</td>
<td>13</td>
<td>Fail</td>
<td>y = 1.03x - 0.63</td>
<td>-0.39</td>
<td>0.19</td>
<td>14</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.03x - 0.44</td>
<td>-0.24</td>
<td>0.20</td>
<td>8</td>
<td>Fail</td>
<td>y = 1.08x - 0.87</td>
<td>-0.28</td>
<td>0.21</td>
<td>12</td>
<td>Fail</td>
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<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 1.00x - 0.27</td>
<td>-0.26</td>
<td>0.22</td>
<td>9</td>
<td>Fail</td>
<td>y = 1.06x - 0.70</td>
<td>-0.30</td>
<td>0.21</td>
<td>15</td>
<td>Fail</td>
</tr>
<tr>
<td>InnovaStar (y)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>vs Premier (x)</td>
<td>y = 0.89x + 0.46</td>
<td>-0.36</td>
<td>0.14</td>
<td>10</td>
<td>Fail</td>
<td>y = 0.89x + 0.43</td>
<td>-0.40</td>
<td>0.13</td>
<td>15</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 0.93x + 0.25</td>
<td>-0.25</td>
<td>0.18</td>
<td>8</td>
<td>Fail</td>
<td>y = 0.93x + 0.21</td>
<td>-0.30</td>
<td>0.15</td>
<td>9</td>
<td>Fail</td>
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<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 0.91x + 0.40</td>
<td>-0.27</td>
<td>0.17</td>
<td>9</td>
<td>Fail</td>
<td>y = 0.90x + 0.37</td>
<td>-0.32</td>
<td>0.16</td>
<td>8</td>
<td>Fail</td>
</tr>
<tr>
<td>Cobas B101 (y)</td>
<td></td>
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</tr>
<tr>
<td>vs Premier (x)</td>
<td>y = 1.06x - 0.48</td>
<td>-0.05</td>
<td>0.24</td>
<td>6</td>
<td>Fail</td>
<td>y = 1.06x - 0.40</td>
<td>0.01</td>
<td>0.24</td>
<td>4</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tina-quant (x)</td>
<td>y = 1.11x - 0.74</td>
<td>0.06</td>
<td>0.23</td>
<td>5</td>
<td>Fail</td>
<td>y = 1.10x - 0.67</td>
<td>0.10</td>
<td>0.23</td>
<td>5</td>
<td>Fail</td>
</tr>
<tr>
<td>vs Tosoh G8 (x)</td>
<td>y = 1.09x - 0.60</td>
<td>0.04</td>
<td>0.17</td>
<td>2</td>
<td>Pass</td>
<td>y = 1.09x - 0.52</td>
<td>0.09</td>
<td>0.17</td>
<td>1</td>
<td>Pass</td>
</tr>
</tbody>
</table>

* Thirty-seven of 40 results need to be within 6% (relative) of the NGSP SRMP to pass certification.
* SEE, standard error of estimate.
* Same measurement principle as investigated POC method.
value of 73 mmol/mol (8.8% DCCT) means that the next Hb A1c value of the patient should be <64 mmol/mol (8.0% DCCT) or >82 mmol/mol (9.6% DCCT) before one can say that there is a significant change in health status of the patient. A difference of 5 mmol/mol (0.5% DCCT) from a previous Hb A1c value is considered by most healthcare professionals as an indication to adjust therapeutic options. As the DCA Vantage is mostly used in the Netherlands at the pediatric unit for young children and teenagers with (typically) increased Hb A1c values, this could potentially lead to overtreatment of the patient. However, this situation is applicable for all patients with increased Hb A1c values.

Precision studies performed on the Afinion with manufacturer-supplied controls showed lower CVs in a previous study than when fresh patient samples were used and were in accord with the CVs we calculated from the duplicates in the EP-9 protocol (4). However, the CVs were still within the acceptance limits.

Fig. 1. Hb A1c results (SI units) for 2 different reagent lot numbers from (A) Afinion, (B) Cobas B101, (C) DCA Vantage, and (D) B-analyst point-of-care instruments compared to the mean Hb A1c results from 3 secondary reference measurement procedures. ——, Line of identity; ———, ± 6%.

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A limitation to this study is that we have not been able to collect fresh patient samples with different Hb variant samples with varying Hb A1c values over the clinically relevant range to investigate whether B-analyst and Afinion had interference from these Hb variants. Also, this part of the study needs to be repeated once Quo-Test, Quo-Lab, and InnovaStar have been recalibrated with fresh whole blood. In the Hb variant interference study, we used the bias obtained in the familiarization study with frozen samples to correct for the bias found in nonvariant samples. In general, caution is advised for all Hb A1c POC instruments and laboratory-based methods in the presence of Hb variants and it is not known whether a particular Hb variant interferes with the method used.

The bias of the B-analyst compared with the mean of the 3 reference measurement procedures was very low (Fig. 1D) but was not in accord with the results in DCCT units (higher results in the high area than in SI units). Further investigation yielded a different master equation between IFCC and NGSP in the software of the B-analyst than that previously published (22). One could argue that using a different master equation than the published master equation would contribute to the standardization of Hb A1c worldwide. Nevertheless, the B-analyst passed
Fig. 2. Hb A\textsubscript{1c} results (SI units) for 2 different reagent lot numbers from (A) InnovaStar, (B) Quo-Lab, and (C) Quo-Test point-of-care instruments compared to the mean Hb A\textsubscript{1c} results from 3 secondary reference measurement procedures. ———, Line of identity; ———, ± 6%.
the NGSP criteria when compared with 3 SRMPs with 2 reagent lot numbers.

The Cobas B101 is new on the market and in general showed good performance in this study with respect to accuracy and reproducibility. However, there is some room for improvement regarding instrument calibration (Fig. 1B). The Cobas B101 passed the NGSP criteria compared with the Tosoh G8 for both reagent lot numbers but failed when compared with the Tina-quant Gen.2 on Integra 800 and the Premier Hb9210. The new NGSP criterion is quite stringent, especially for the nondiabetic patient range. Thirty-seven of 40 results need to be within 6% (relative) of the NGSP SRMP to pass certification. Six percent of a value of 5.0% DCCT is 0.30%, which is not much considering that some patients have an individual matrix effect with a certain method that is unrelated to the bias observed between the 2 methods. This was not the case with the Cobas B101, because most of the samples in the nondiabetic patient range were too low, which would suggest a calibration problem and not an individual patient matrix effect. The Cobas B101 showed interference from Hb AE. This is a problem if this method is used in a part of the world where the prevalence of Hb AE is high and patients are not routinely screened for this variant. The patients will get an Hb A1c value that is falsely high with possibly a more stringent therapy than is necessary.

The findings for Hb F interference found in this study were consistent with those from a previous study (23). The reason for the proportional interferences with the affinity methods is probably a result of the lower glycation rate for Hb F compared with Hb A and the lack of recognition of glycated Hb F by the Hb A1c antibody with the immunoassay methods. It is reasonable to generalize this to all affinity- and immunoassay-based methods.

It would be in the interest of all patients with diabetes to screen all patients at the time of diagnosis for hemoglobinopathy and thalassemia, not only to see if this variant might interfere with the Hb A1c method but also to know if the Hb variant is associated with a shorter turnover of red blood cells, which leads to a falsely low Hb A1c value.

The American Diabetes Association concluded in 2011 that POC Hb A1c assays were not sufficiently accurate at that time to use for diagnostic purposes and calibrated with IFCC secondary reference material. This is not sufficient. However, in 2014, the American Diabetes Association concluded that although POC Hb A1c assays may be NGSP certified, proficiency testing is not mandated for performing the test, so use of these assays for diagnostic purposes may be problematic. In reviewing CAP survey results from recent years, it seems that not all POC instruments should be excluded for use in the diagnosis of diabetes because Afinion, DCA 2000, and DCA Vantage showed excellent results, even better than some laboratory-based methods. This was also the conclusion of an article recently published in clinical chemistry on the basis of the results of many years of an external quality scheme in Scandinavia. There are no barriers for the manufacturers of POC instruments to attempt to gain FDA approval to use their instrument for the diagnosis of diabetes. However, in addition to FDA approval, we think that proficiency testing should be mandated for users of POC assays to ensure quality.

The EP-9 protocol is probably not the best protocol to decide whether a POC instrument can be used for the diagnosis of diabetes, as the number of samples around the cutoff value of 48 mmol/mol (6.5% DCCT) is not sufficient. However, in an attempt to strengthen our argument, we used 3 SRMPs and calibrated with IFCC secondary reference material; therefore, the values are as close as possible to the true value and are likely more reliable than comparing

| Table 3. MDP analysis of 48 mmol/mol (95% CI) compared with the mean of the 3 SRMPs. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Lot number 1    | Lot number 2    |                  |                  |                  |                  |
| B-Analyst        | 48.1 (47.8–48.4)| 47.7 (47.4–48.0)|                  |                  |                  |                  |
| DCA Vantage      | 46.9 (46.4–47.4)*| 47.9 (47.4–48.3)|                  |                  |                  |                  |
| Afinion          | 47.5 (47.0–48.0)| 48.2 (47.8–48.6)|                  |                  |                  |                  |
| Quo-Test         | 45.1 (44.7–45.6)*| 45.1 (44.7–45.5)*|                  |                  |                  |                  |
| Quo-Lab          | 44.8 (44.3–45.3)*| 44.1 (43.6–44.5)*|                  |                  |                  |                  |
| InnovaStar       | 45.4 (45.0–45.7)*| 44.9 (44.6–45.3)*|                  |                  |                  |                  |
| Cobas B101       | 47.5 (47.0–48.0)| 48.2 (47.7–48.7)|                  |                  |                  |                  |

* Significantly different from mean SRMP.

| Table 4. Mean relative difference (%) of the common Hb variants (n = 5 per variant) compared to the assigned value. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Hb AS           | Hb AC           | Hb AD           | Hb AE           | Elevated A2     | Hb F*           |
| DCA Vantage      | 3.7             | 6.9             | 1.6             | 3.6             | −1.4            | −12.3           |
| Cobas B101       | −2.1            | −1.6            | 3.4             | 17.1            | 2.1             | −11.1           |
| Quo-Test         | −5.3            | −3.8            | −3.6            | 2.5             | 0.4             | −12.6           |
| Quo-Lab          | −6.0            | −1.7            | −0.9            | 5.3             | −3.6            | −11.2           |
| InnovaStar       | 5.5             | 4.4             | −3.2            | 5.9             | 3.0             | −18.8           |

* HbF percentages of 3.2%, 4.2%, 6.9%, 12.0%, and 18.3%.
with just 1 SRMP, MDP analysis showed that Afinion, DCA Vantage, Cobas B101, and B-analyst may be suitable for the diagnosis of diabetes differed from the mean of the SRMPs (Table 3), but clinically speaking, the difference was minimal.

In conclusion, Afinion, B-analyst, and Cobas B101 met the generally accepted performance criteria for Hb A1c. DCA Vantage met the criteria in the diagnostic range but showed a high CV > 64 mmol/mol (8.0% DCCT), which is the lowest Hb A1c value of the 95% CI of 73 mmol/mol (8.8% DCCT). Users of the DCA Vantage should be informed not to change therapy on the basis of a small difference between two consecutive Hb A1c values when the Hb A1c value is > 64 mmol/mol (8.0% DCCT). Quo-Test, Quo-Lab, and InnovaStar are potentially good methods but need to be calibrated and certified with fresh patient samples instead of frozen material. Cobas B101 should not be used in regions where the prevalence of Hb AE is high unless the patient has been screened for this hemoglobin variant and found to be negative.

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