Measurement of Hemoglobin A\textsubscript{1c} from Filter Papers for Population-Based Studies

David A. Egier, Judy L. Keys, S. Kim Hall, and Matthew J. McQueen\textsuperscript{1,2,3*}

**BACKGROUND:** Stability and transport challenges make hemoglobin (Hb) A\textsubscript{1c} measurement from EDTA whole blood (WB) inconvenient and costly for large-scale population studies. This study investigated Hb A\textsubscript{1c} measurement from WB blotted on filter paper (FP) in a Level I National Glycohemoglobin Standardization Program (NGSP)-accredited laboratory.

**METHODS:** Three Bio-Rad Variant\textsuperscript{TM} II HPLC instruments and WB and FP specimens were used. Precision, accuracy, linearity, and readable total area of the 6.5-min (β-thalassemia method) Variant II HbA\textsubscript{2}/HbA\textsubscript{1c} Dual Program were assessed. Hb A\textsubscript{1c} stability was measured using in-house FP QC samples. The INTERHEART (a study of the effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries) and CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) studies provided chromatographs for morphometric analyses and interoperator variability experiments. Statistical analyses were performed to assess long-term sample stability, WB vs FP agreement, and significance of Hb A\textsubscript{1c} peak integration.

**RESULTS:** Intra- and interassay CVs were ≤2.00%. Total area counts between 0.8 and 5.5 × 10\textsuperscript{6} μV/s produced accurate Hb A\textsubscript{1c} results. The regression equation for agreement between WB(x) and FP(y) was as follows: \[ y = 0.933x + 0.4 \quad (n = 85). \] FP QC samples stored at −70 °C and tested over approximately 3 years yielded CVs of 1.72%–2.73% and regression equations with slopes of −1.08 × 10\textsuperscript{−4} to 7.81 × 10\textsuperscript{−4}. The CURE study, with better preanalytical preparation, achieved a 97% reportable rate, and the reportable rate of the INTERHEART study was 85%.

**CONCLUSIONS:** The FP collection method described provided accurate, robust, and reproducible measurement of Hb A\textsubscript{1c} using the Bio-Rad Variant II HPLC autoanalyzer when FP specimens were prepared according to standardized protocols, and analyses were performed in an NGSP-certified laboratory, supporting the use of FP collection cards in large multinational studies.

Diabetes affects >285 million people globally (112% increase since 1995); and this number is projected to increase to almost 440 million by 2025 (1, 2). Correlation between hyperglycemia and complications such as retinopathy and neuropathy was established by the Diabetes Control and Complications Trial (DCCT)\textsuperscript{4} (1983–1993) (3, 4), and the cardiovascular disease relationship was established by the Epidemiology of Diabetes Interventions and Complications study (1993 onward) (5). Because these complications are the leading causes of morbidity and mortality in people with diabetes and are reduced when hemoglobin A\textsubscript{1c} (Hb A\textsubscript{1c}) is <7%, stringent glycemic monitoring and control is essential (6). Hb A\textsubscript{1c} measurement is used with other glucose tests in screening for diabetes (7), and Hb A\textsubscript{1c} monitoring influences clinical treatment decisions.

Hb A\textsubscript{1c} is the amino-terminal nonenzymatic glycation (on amino-terminal valine residues of the β-chain) product of Hb A and depends on the concentration of blood glucose and the lifespan of circulating red blood cells (approximately 120 days) (8, 9). Hb A\textsubscript{1c} levels (expressed as a percentage of total Hb A) reflect long-term blood glucose concentrations and thus the efficacy of glycemic control (10, 11) over the prior 2–3 months, 50% of which is representative of the previous month, 25% of the previous 2 months, and 25% of the previous 3 months (12, 13).

Whole blood (WB) venous samples collected by venipuncture into EDTA Vacutainer Tubes are used most commonly for Hb A\textsubscript{1c} measurement, and transportation to a central laboratory in large-scale...
population-based studies is costly. The stability of Hb A1c is questionable when there are variable and extended periods between collection and analysis (14, 15). Blood sample collection onto filter paper (FP) has been implemented in epidemiologic studies (16) and significantly decreases transportation costs and limits the challenges of shipping dangerous goods in large-scale multinational population studies. Previous work showed that Hb A1c FP samples are stable and provide reliable, reproducible values after 5–7 days at room temperature, 10 days at 4–6 °C, and several months at /H11002 70 °C (17–20). This investigation examined FP collected in 78 countries representing every region of the world for suitability to measure Hb A1c.

**Materials and Methods**

**ANALYTICAL INSTRUMENTS AND Hb A1c MEASUREMENT**

Three Bio-Rad Variant II (www.biorad.com) ion-exchange HPLC instruments were used with the 6.5-min (β-thalassemia method) Variant II HbA2/HbA1c Dual Program to measure Hb A1c in WB and extracted FP samples. Prior investigation using the 1.5-min (Turbo) and 3-min Variant II programs resulted in insufficiently separated chromatograph elution peaks. The instrument autoinjects samples into an analytical cartridge, which separates hemoglobins on the basis of ionic interaction with the cartridge material. Eluted hemoglobins pass through the detection station, where changes in absorbance are read at 415 nm (background corrected at 690 nm). Clinical data management software analyzes the raw absorbance data and uses an exponentially modified gaussian algorithm to calculate areas for Hb A2, Hb F, and Hb A1c.

Hb A1c was measured from WB and from drops of the same WB applied to FP. The Variant II instruments were calibrated daily at the beginning of the first analytical run with dual-level (β-Thal CalSet; Bio-Rad) standardized to the DCCT. Bio-Rad A2/F controls were tested at the beginning of each run, and in-house FP QC samples (blotted from WB and stored at /H11002 70 °C) were tested at the beginning and end of each analytical run. The FP and WB samples and Bio-Rad A2/F controls were prediluted in 1 mL Bio-Rad hemolyzing buffer. A 3/16-inch disk was punched from each FP sample into the extraction buffer; 30 min was allowed for blood to elute into solution at room temperature, which was followed by removal of the disk. Five microliters of each WB sample and Bio-Rad control was added to 1 mL of hemolyzing buffer. Vials were inverted to mix thoroughly before analysis. Each resulting chromatograph was initially screened by using acceptance/repeat/rejection criteria developed within the Clinical Research and Clinical Trials Laboratory (CRCTL) (Table 1).

**INSTRUMENT/METHOD VALIDATION**

**Intraassay precision.** Intraassay precision was determined by using in-house FP samples stored at −70 °C (normal approximately 5.6% and high approximately 8.0%). Ten FP hemolysates of each level were prepared and analyzed on each of 3 instruments on each of 3 days. Precision was determined as CV%: [(SD/mean) × 100], calculated from the mean and SD of each sample on each instrument each day.

**Table 1. Criteria used to assess chromatograph acceptability.**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Repeat/rejection details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low area</td>
<td>&lt;800 000 μV/s</td>
</tr>
<tr>
<td>High area</td>
<td>&gt;5 500 000 μV/s</td>
</tr>
<tr>
<td>Poor integration</td>
<td>Hb A1c integration peak does not properly integrate area under elution peak</td>
</tr>
<tr>
<td>Inadequate separation</td>
<td>Inadequately separated LA1c and Hb A1c elution peaks</td>
</tr>
<tr>
<td>No Hb A1c peak</td>
<td>No integration peak present for Hb A1c</td>
</tr>
<tr>
<td>Blank chromatograph</td>
<td>No elution pattern present</td>
</tr>
<tr>
<td>Another peak &gt; Hb An peak</td>
<td>Hb An peak must be the largest</td>
</tr>
<tr>
<td>Variant/unknown peak interference</td>
<td>Large variant/unknown peak present at retention time similar to that of Hb A1c and interferes with Hb A1c elution</td>
</tr>
<tr>
<td>Abnormal baseline curvature</td>
<td>Dramatic changes in baseline curvature</td>
</tr>
<tr>
<td>Software exception</td>
<td>Error code; Bio-Rad Clinical Data Management software does not properly compute a result</td>
</tr>
<tr>
<td>High Hb A1c retention time</td>
<td>&gt;1.03 min</td>
</tr>
<tr>
<td>Low Hb A1c retention time</td>
<td>&lt;0.83 min</td>
</tr>
</tbody>
</table>
Interassay precision. Interassay precision was assessed by using freshly prepared Bio-Rad WB control samples, in-house FP QC samples (normal approximately 5.6% and high approximately 9.0%), in-house FP samples (normal approximately 5.6% and high approximately 8.0%), and 5 WB samples (WB1–5), tested as WB and WB blotted on FP (in-house FP QC samples and WB1–5 samples stored at −70 °C for 1 year and 8 months, respectively). Hemolysates were prepared daily and tested on each of 3 days. FP samples, WB1–5 samples, and Bio-Rad WB controls were tested twice daily; in-house FP QC samples and WB1–5 FP samples were tested once daily on each instrument for 3 days. The mean, SD, and CV% for each specimen type were calculated across all instruments over 3 days.

Accuracy. Accuracy was calculated from National Glycohemoglobin Standardization Program (NGSP) quarterly monitoring and annual accreditation testing. Annual accreditation accuracy testing used 40 unique samples, 8 analyzed in duplicate per day (testing spanned 5 days). For quarterly accuracy monitoring, 10 samples were tested once daily for 2 days. Our accuracy was assessed as the fractional error [(%\(Hb\ A_1c\) FP − %\(Hb\ A_1c\) NGSP)/%\(Hb\ A_1c\) NGSP] between the mean we obtained for a sample and that measured by the NGSP reference laboratory.

Readable area range. A WB sample with an \(Hb\ A_1c\) of 6.1% was diluted 1 in 2 with diluent. Seven serial dilutions of this sample in hemolyzing buffer (1 in 4 to approximately 1 in 20) were used to determine the range of readable area that produced a reliable \(Hb\ A_1c\) result (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue4). Hemolysates were blotted on FP, eluted, and analyzed to determine the following: \(Hb\ A_1c\) peak area, percent \(Hb\ A_1c\), and the acceptability of the chromatograph and reported \(Hb\ A_1c\) value.

Linearity. Linearity was evaluated by using 22 WB samples, each prepared as 10 dilutions with homologous plasma (see Table 1 in the online Data Supplement) to determine whether %\(Hb\ A_1c\) is affected by total hemoglobin concentration. Each dilution of each sample was blotted onto FP, air-dried, frozen overnight at −70 °C, thawed, and analyzed.

WB vs FP method comparison
Eighty-five routine clinical WB specimens were selected on the basis of an initial \(Hb\ A_1c\) result, with emphasis on the clinically relevant \(Hb\ A_1c\) range (approximately 5.5% to 8.5%), stored at 4 °C, blotted on FP, and analyzed within 96 h of sample collection. Each FP and matching WB sample were analyzed on the same run. Passing–Bablok and Bland–Altman method comparison analyses were performed to evaluate bias between sample types.

FP stability
FP QC samples prepared in-house in the normal (approximately 5.6% and 5.3%) and high (approximately 9.0% and 9.7%) \(Hb\ A_1c\) range were stored at −70 °C and tested at the beginning and end of every analytical run over approximately 3 years. Sample stability was assessed by using Deming linear regression and calculated as the CV% of all measures on each instrument for each QC.

Statistical methods
Passing–Bablok and Deming linear regression analyses and Bland–Altman bias testing were performed in Analyse-it Standard Edition for Microsoft Excel (Analyse-it Software, www.analyse-it.com). Kruskal–Wallis, Dunns, and further Bland–Altman testing were performed by using GraphPad Prism 5 (GraphPad Software, www.graphpad.com). Statistical significance was defined as \(P < 0.05\).

Sample collection for epidemiologic studies
Approximately 700 collection centers in 78 countries followed standardized sample collection and handling protocols provided by the CRCTL for both the INTERHEART (a study of the effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries) (21) and Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) (22) studies. Research ethics review boards at each local site approved the study protocols, and all participants provided informed consent before specimen collection. When venipuncture was performed for collection of clinical specimens, an additional tube of WB was collected in an EDTA Vacutainer Tube (Becton Dickinson, www.bd.com/ca) and mixed by inversion, and approximately 50 µL (1 drop) was applied to FP collection cards (Roche, www.roche.com). Each FP was allowed to air dry for 2 h, sealed in an individual resealable plastic bag, and frozen locally at −20 °C for ≤3 months (based on in-house stability data) or −70 °C for ≤6 months. A total of 15 855 FPs were shipped frozen on ice packs to the CRCTL and stored at −70 °C until analysis.

Morphometric analysis of borderline chromatographs
In an attempt to improve objectivity in the evaluation of peak integration, 100 chromatographs of varying quality were reviewed 3 times by 5 operators (3 experienced, 2 naive). Chromatographs were classified on the basis of acceptability of \(Hb\ A_1c\) peak integration; those...
defined by ≥4 operators as accepted or rejected were classified accordingly, and images were deemed borderline if multiple operators did not consistently accept/reject a chromatograph when shown it 3 times in a blinded trial. This resulted in a subset of 25 “borderline” chromatographs. Morphometric analysis of this subset (Adobe Photoshop 7, www.adobe.com) determined the area of the integration peak, nonintegrated area (between the integration peak and the elution peak), and the total area under the elution peak. Area measurements were restricted to the region within the Hb A1c retention time window (defined by the instrument as 0.83 < t < 1.03 min on the x-axis). Maximum integration peak height and total width of the bell-shaped curve (trough-to-trough) were also measured.

Subsequent analysis revealed that the 25 chromatographs consistently possessed poorly integrated Hb A1c peaks. From these, 14 borderline chromatographs displaying only the “poor integration” trait (without other confounding traits described in Table 1) were selected to further quantify this subjective feature. To estimate interchromatograph error associated with the morphometric analysis, area measurements were repeated 10 times on a single chromatograph and CV% was calculated for integrated, nonintegrated, and total areas.

Results

BIO-RAD VARIANT II 6.5-MIN PROGRAM VALIDATION

In-house prepared FP samples at both normal (approximately 5.6%) and high (approximately 8.0%) Hb A1c values yielded excellent intraassay (CV% ≤1.84% and 1.29%, respectively) and interassay (CV% ≤1.60% and 1.23%, respectively) precision. Interassay precision testing across all QC samples (on all instruments) generated CV% of ≤2.00%. Table 2 provides a summary indicating that all data from all 3 instruments are consistent with excellent performance. The instruments performed well, meeting the intralaboratory imprecision specifications recommended by Sacks et al. (23) and Bio-Rad (<3% and ≤4%, respectively).

Accuracy assessment from NGSP accreditation monitoring across the Hb A1c range of 4.45% to 13.5% revealed an increasing negative bias (range of 0.02% to −0.73%; fractional error range of 0.0034 to −0.0602; see Table 2 in the online Data Supplement) with DCCT-referenced Hb A1c values. When focused on the clinically significant range (Hb A1c ≤8.5%), the mean negative bias was −0.1% (fractional error = −0.0128). This level of accuracy meets the level I standard for NGSP accreditation [accuracy, lower 95%, upper 95% (±0.75%)].

Readable area range experimentation demonstrated consistently acceptable Hb A1c results and chromatograph elution patterns when total area of analysis was between 0.8 and 5.5 × 10⁶ μV/s (compared to Bio-Rad’s suggested range of 1.5–3.5 × 10⁶ μV/s).

Linearity testing of FP blotted with WB samples prediluted with homologous plasma yielded Deming regression equations with a mean slope of −3.83 × 10⁻³ (range −5.7 × 10⁻² to 7.0 × 10⁻²). The mean fractional error [(%Hb A1c of diluted sample − %Hb A1c of neat sample)/%Hb A1c of neat sample] between each dilution and its neat sample was −0.0026 (mean absolute fractional error of 0.0108) with a maximum of 0.0488, indicating that sample values were virtually unaffected by dilutions as great as 2 in 5.

WB VS FP METHOD COMPARISON

All chromatographs for WB and matching FP were acceptable according to the criteria in Table 1. A Passing–Bablok agreement plot and Bland–Altman method comparison for 85 WB samples and matching FP samples revealed little difference between the sample types (FP Hb A1c) = 0.933(WB Hb A1c) + 0.4] (Fig. 1A) with a slight negative bias [percent difference = −1.66% (1.94%)]) (Fig. 1B). However, when the comparison of FP to WB was restricted to the 51 samples in the clinically significant range (≤8.5%), linear regression indicated less negative bias [FP Hb A1c] =

Table 2. Bio-Rad Variant II 6.5-min (β-thalassemia method) HbA2/HbA1c Dual Program interassay validation data.a

<table>
<thead>
<tr>
<th>Material</th>
<th>Sample type</th>
<th>Mean %Hb A1c</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad control 1</td>
<td>WB</td>
<td>5.56</td>
<td>0.098</td>
<td>1.77</td>
</tr>
<tr>
<td>Bio-Rad control 2</td>
<td>WB</td>
<td>9.86</td>
<td>0.098</td>
<td>1.00</td>
</tr>
<tr>
<td>FP control 1</td>
<td>FP</td>
<td>5.62</td>
<td>0.067</td>
<td>1.19</td>
</tr>
<tr>
<td>FP control 2</td>
<td>FP</td>
<td>9.00</td>
<td>0.071</td>
<td>0.79</td>
</tr>
<tr>
<td>FP sample 1</td>
<td>FP</td>
<td>5.58</td>
<td>0.065</td>
<td>1.16</td>
</tr>
<tr>
<td>FP sample 2</td>
<td>FP</td>
<td>8.00</td>
<td>0.077</td>
<td>0.96</td>
</tr>
<tr>
<td>WB 1</td>
<td>WB</td>
<td>5.11</td>
<td>0.102</td>
<td>2.00</td>
</tr>
<tr>
<td>FP</td>
<td></td>
<td>5.17</td>
<td>0.058</td>
<td>1.12</td>
</tr>
<tr>
<td>WB 2</td>
<td>WB</td>
<td>5.45</td>
<td>0.099</td>
<td>1.81</td>
</tr>
<tr>
<td>FP</td>
<td></td>
<td>5.48</td>
<td>0.067</td>
<td>1.22</td>
</tr>
<tr>
<td>WB 3</td>
<td>WB</td>
<td>5.78</td>
<td>0.088</td>
<td>1.52</td>
</tr>
<tr>
<td>FP</td>
<td></td>
<td>5.79</td>
<td>0.078</td>
<td>1.35</td>
</tr>
<tr>
<td>WB 4</td>
<td>WB</td>
<td>8.50</td>
<td>0.141</td>
<td>1.66</td>
</tr>
<tr>
<td>FP</td>
<td></td>
<td>8.21</td>
<td>0.060</td>
<td>0.73</td>
</tr>
<tr>
<td>WB 5</td>
<td>WB</td>
<td>10.84</td>
<td>0.134</td>
<td>1.23</td>
</tr>
<tr>
<td>FP</td>
<td></td>
<td>10.06</td>
<td>0.053</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*Mean, SD, and CV% calculated across all 3 Variant II instruments.
0.941(WB Hb A1c) + 0.353; bias, percent difference = −0.83 (1.8)%.

**FP STABILITY**

In-house FP QC samples stored at −70 °C showed virtually no degradation over 3 years (CV% 1.72–2.73) (see Table 3 in the online Data Supplement). Deming linear regression analysis for each control yielded slopes with a range of $1.08 \times 10^{-4}$ to $7.81 \times 10^{-4}$ (Fig. 2). Chromatographs from these samples had similar elution patterns and consistent total area counts.

**LARGE-SCALE STUDY APPLICABILITY**

To validate the applicability of the decision rules (Table 1), we reviewed our Hb A1c data from 2 major multinational studies, INTERHEART (21) (n = 11 127) and CURE (22) (n = 4728). This review was undertaken to assess the rate of FP sample repeat (following a single test) as well as the number of nonreportable samples. Evaluation of the chromatographs from these studies revealed an initial repeat rate (based on a single test) of 16.57% and 13.64%, respectively, indicating approximately 85% of samples collected under field conditions were reported with confidence on a single test. After repeat testing, 84.7% of INTERHEART and 96.8% of CURE specimens were reported with confidence.

**MORPHOMETRIC ANALYSIS OF BORDERLINE CHROMATOGRAPHS**

Morphometric analysis of 42 chromatographs (14 accepted, 14 borderline, and 14 rejected) revealed striking differences between these 3 groups in Hb A1c peak integration relative to the total area beneath the elution peak (Fig. 3). Accepted images had significantly lower ($P < 0.01$) percentages of nonintegrated Hb A1c peak area [20.54% (12.17%)] than high mean percent nonintegrated areas [44.16% (7.28%)] for those classified as rejected (Fig. 3). The mean percent nonintegrated area for the “borderline” subset of chromatographs was intermediate [35.40% (3.28%)] to and significantly different ($P < 0.05$) from the accepted and rejected groups. The variation for 10 measurements of total integrated, nonintegrated, and total area on a single chromatograph was minimal (CVs ≤1.68%), indicating the automated area-counting tool provided reproducible area (pixel) counts and is a valid means for data acquisition and assessment of integration (see Table 4 in the online Data Supplement).

The criterion “inadequate separation” could not be quantified by using the morphometric tool. The spectrum for the degree of separation criteria is illustrated in Fig. 4 by chromatographs of 3 different specimens. The 3 chromatographs exhibit distinct differences in the degree of labile Hb A1c (LA1c) and Hb A1c separation, such that the result illustrated in Fig. 4A is acceptable, the result in 4B would be repeated/reevaluated, and the result in 4C would be rejected according to the criteria listed in Table 1.

**Discussion**

Numerous methods exist for the determination of WB Hb A1c, including column chromatography, electrophoresis and isoelectric focusing, and colorimetric and immunoassays (24–26). Ion-exchange HPLC methods...
allow Hb A1c determination without interference from its Schiff base (LA1c) and can be used for variant screening (27, 28). Automated HPLC instruments allow rapid and reproducible analysis of samples, appropriate for large population-based studies.

Previous evaluation of the Bio-Rad Variant II yielded intra- and interassay precision of <5% (28) and demonstrated the utility and validity of the dual program for measurement of Hb A1c from routine clinical WB samples (29, 30). We identified 2 limitations affecting our large-scale epidemiologic studies using FP samples: a high repeat rate for samples collected under variable conditions and a negative bias in the high end (>8.5% Hb A1c) of the DCCT range for FP samples relative to WB.

Earlier (17) and more recent (31) reports indicate that Hb A1c analysis using FP samples yields comparable results to those acquired using WB. Our study extends these findings and validates the use of FP samples collected under field conditions in 78 countries from patients enrolled in large, multinational, population-based studies. The Bio-Rad Variant II instrument, using the 6.5-min (β-thalassemia) Variant II HbA2/HbA1c Dual Program, is a superior method for Hb A1c measurement in a central laboratory for FP blotted with WB.

Our data quantify the Bio-Rad Variant II 6.5-min (β-thalassemia) HbA2/HbA1c Dual Program accuracy, precision, and robustness. Intraassay precision was consistently <2%, and >90% of interassay CV% values were <2%. Dilution experiments indicated consistent and reproducible %Hb A1c results across a broad range of sample dilutions, and the reportable total area range (µV/s) on the instrument

![Fig. 2. Long-term stability data for in-house prepared FP quality controls.](image)

Both normal FP controls (A and C) and high FP controls (B and D) were stable at −70 °C for up to 3.25 years. Solid center lines representing Deming regression lines are flanked by curves indicating proportional variance. Outermost lines are 95% CIs.
accommodated both dilute and concentrated FP eluates, as reported by Higgins et al. (28). This is important because our experience with FP collection on a global scale indicates some WB samples settle before blotting on FP, resulting in a concentrated sample drawn from the erythrocytes in the bottom of the tube, or a dilute sample drawn near the top of the tube.

NGSP accreditation monitoring of the 6.5-min (β-thalassemia) Variant II HbA2/HbA1c Dual Program on the Bio-Rad Variant II indicated a slight negative bias in %Hb A1c results from FP samples in comparison to their NGSP reference value (−0.1%; mean fractional error of −0.0128 within the clinically significant range). The CRCTL has held level I NGSP accreditation on both WB and FP Hb A1c samples for the past 5 years, confirming the long-term accuracy and precision of this method.

WB Hb A1c samples frozen at −70 °C have been shown to provide reliable results after a decade of storage (15). Earlier work indicated FP samples remain stable at −70 °C for several months (17). Our data indicate that FP blotted with WB and stored at −70 °C maintained sample integrity and yielded CVs ≤2.73% over approximately 3 years, supporting research facilities and/or biorepositories storing samples at −70 °C over many years.

Large-scale/multinational population-based studies present difficulties not normally encountered during routine clinical analysis. Although standardized protocols are provided to sample collection sites, samples collected in global studies may be subjected to potentially degenerative effects of harsh preanalytical conditions. Nevertheless, the Bio-Rad Variant II generated reportable values for approximately 83% and approximately 86% for INTERHEART and CURE, respectively, of 15 855 FP samples on the first test. INTERHEART samples were frequently rejected for issues related to the quality of sample preparation and preanalytical sample degradation (i.e., Hb A1c shouldering, high LA1c, very low area counts), whereas CURE specimens were rejected for instrument pro-
In summary, the data presented in this report validate both the collection of WB on FP for Hb A1c determination in large-scale population studies and testing of these FP samples on the Bio-Rad Variant II using the 6.5-min (β-thalassemia) Variant II HbA2/HbA1c Dual Program in a level I NGSP-accredited laboratory. The negative bias in FP results compared to WB is negligible and does not affect clinical decisions (Fig. 1). The utility of FP collection under field conditions makes worldwide sample collection for Hb A1c testing feasible. However, it is imperative that personnel in the field receive adequate training and understand the importance of consistent collection, handling, freezer storage, and shipment practices, to avoid high nonreportable rates at analysis. In addition, we anticipate that our approach to the quantification and visual representation of qualitative and highly subjective chromatograph traits deemed “reasons for repeat/rejection” will reduce interoperator decision-making variability and improve the analysis and reporting of Hb A1c values.

References

Hb A1c Measurement from Filter Papers in Population Studies


