Correlation of Rapid HbA1c Test with High Performance Liquid Chromatography Measurements: A MetroNet Study

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Abstract

**Background:** Glycated hemoglobin (HbA1c) results vary based on the analytical method. Use of rapid HbA1c testing methodology holds the promise of more efficient patient care, and improved HbA1c levels. Our objective was to test the feasibility of introducing a rapid HbA1c methodology into busy family practice centers and to calculate the correlation between the rapid HbA1c test and the traditional high performance liquid chromatography (HPLC) technique.

**Methods:** The study was conducted at two family practice centers (FPCs) in southeast Michigan. Consecutive diabetic patients 18 years of age and older having blood samples drawn for routine HPLC analysis of HbA1c were asked to provide a capillary blood sample for in-office rapid testing with the BIO-RAD Micromat II. Data were analyzed separately by site because the FPCs used different HPLC methodologies.

**Results:** 147 paired samples were available for analysis (n = 73 from one site, and n = 74 from the other site). The Pearson correlation of the Micromatt II with an ion-exchange HPLC was 0.713 (p < 0.001). The Micromatt yielded a mean HbA1c of 6.91%, which was lower than the 7.23% from the ion-exchange HPLC analysis (p < 0.001). The correlation of the Micromatt II with the boronate-affinity HPLC was 0.788 (p < 0.001). The Micromatt provided a mean HbA1c of 6.54% which lower than the 7.75% from the boronate-affinity HPLC (p < 0.001). Medical staff found the same-visit measurement difficult to perform due to the amount of dedicated time required for the test.

**Conclusions:** The correlation coefficient obtained in the busy clinical practice setting was lower than the 0.98 reported by the manufacturer. This might be due to the variability introduced by the multiple users of the machine. The amount of dedicated
time required to perform the assay may limit its usefulness in a busy clinical practice. Before introducing a rapid HbA1c methodology, clinicians should compare the rapid results to their current method of analysis.
Background

The percent HbA1c of glycated hemoglobin provides an estimate of blood glucose levels over a 3-4 month period. The HbA1c level is used for patient education and counseling, for feedback about diabetic control, to improve patient motivation, and to monitor management; thus its measurement should be optimally accurate and precise [1]. However, to date, there is no international standard for determining HbA1c [2-4], and various methodologies are commercially available. Tran [1] determined the physiological (changes over time between measurements) and analytic variation of two widely used laboratory assays, one a high performance liquid chromatography (HPLC) method, and the other an immunoassay [1]. The coefficient of variation (CV) for the HPLC was 2.6%. The 5.1% CV of the immunoassay method exceeded physiologically established limits of 2-3%, and those of the National Glycohemoglobin Standardization Program (3-4%).

Hosseini et al. [5] reported a relative ranking of assays to result in a normal HbA1c level by using the same patient’s blood tested with five assays, each of which used a different method. They found that glycated hemoglobin results vary widely, with some assays consistently more likely to result in a “normal glycated hemoglobin” level than other assays and consequently have differing implications for an individual patient to achieve a HbA1c level within the normal range. Ogawa [6] reported a case series where HbA1c was underestimated in the measurement by HPLC which excluded glycated abnormal hemoglobin [6]. These findings illustrate the potential usefulness for clinical practitioners to evaluate the performance of their method for determining HbA1c.
Recent developments in medical technology allow clinicians to determine HbA1c test results during a patient’s office visit. Several manufacturers offer an assay that can be performed by trained medical personnel and yield HbA1c results in five to ten minutes. We found only a few reports of the performance of such rapid tests used at the point of care [7, 8], and one study was conducted by the test manufacturer [9].

The objective of this pilot study was to test the feasibility of introducing a rapid HbA1c methodology into busy family practice centers and to compare the results obtained from a point-of-care test with a laboratory-based (HPLC) technique. Specifically, our purpose was to determine: 1) if a specific rapid HbA1c methodology was accepted by medical support staff in two busy family practice centers (FPCs); and 2) how rapid HbA1c results compared with the standard laboratory HPLC methodology.

Methods

Study Design

Patients were recruited for this cross-sectional study from two FPCs that are members of MetroNet, a metropolitan Detroit practice-based research network. At both sites, HbA1c analysis is routinely performed at an outside laboratory using a HPLC methodology on venipuncture samples. Physicians, medical assistants, and research assistants identified consecutive diabetic patients 18 years of age and older whose physicians ordered HbA1c analysis. The study was explained to these eligible patients and informed consent obtained from those who wished to participate.

After patients were enrolled, a finger-prick blood sample was collected for in-office HbA1c testing with the BIO-RAD Micromat II. Since the BIO-RAD Micromat II is compatible with capillary, venous, and EDTA anti-coagulated blood samples, aliquots
of these types were also acceptable for analysis. Research and medical staff were
instructed to use finger-prick capillary samples whenever possible, but venous samples
from the blood draw apparatus, or a drop of blood from the EDTA tube was substituted
when necessary.

The data collected included patient name, study site, the person performing rapid
HbA1c analysis, the date, and the rapid HbA1c result. Physicians were blinded to rapid
HbA1c results, and relied on the laboratory HPLC analysis to make treatment decisions
during the study period. One FPC laboratory used the Primus Model 386 incorporating
boronate affinity HPLC. The other laboratory used the Tosoh A1c 2.2 Plus for ion-
exchange HPLC for analysis.

The BIO-RAD Micromat II, which provides results in approximately 5 minutes,
incorporates an affinity chromatography method that measures the percent glycated
hemoglobin in the sample. According to the manufacturer, the analyzer then uses a
factory-set algorithm to deliver an HbA1c result which is calibrated to the
recommendations of the Diabetes Control and Complications Trial and is traceable to the
National Glycohemoglobin Standardization Program, an internationally accepted method
of standardization. BIO-RAD representatives provided an in-service to help familiarize
staff in the use and operation of the analyzer.

Each HbA1c analysis with the Micromatt II requires a single test cartridge, which
consists of several tubes with reagents that are mixed and decanted into a collection
reservoir for measurement. After a test cartridge has been placed into the Micromat II, a
20 microliter blood sample is added to the first tube. This initiates a series of aliquot
additions and incubation steps. In total there are four decanting steps followed by four
These incubations require a total time of 230 seconds and range from 40 seconds to 80 seconds in length. Quality control procedures were carried out as outlined in the Micromat II instruction manual. Controls and standards were run per the manufacturer’s recommendation; results were always acceptable.

**Analytic Strategy**

Data were analyzed separately by site because the two FPCs used different HPLC methodologies. To evaluate the performance of the BIO-RAD Micromat II, Pearson correlations were conducted using the laboratory HPLC results as the standard. The mean absolute difference between the sample groups was calculated to test the hypothesis that group means are equal ($\alpha = 0.05$), using a two-sided paired t-test.

**Results**

One hundred fifty-six patients were enrolled into the study (75 from one FPC, and 81 from the other FPC). Nine different medical staff performed the rapid HbA1c testing. The data from nine patients were omitted: eight had missing laboratory HPLC results, and one result was out of the precision range of the machine (HbA1c = 18.1%). Therefore, 147 paired samples were available for analysis, 73 from one site and 74 from the other.

Considering first the data from the site that used ion-exchange HPLC ($n = 73$), we found a significant correlation with the Micromat II results (Pearson $r = 0.713$, $p = 0.001$). The range of values was from 2.3% to 12.70%. The HPLC method yielded a mean HbA1c value ($7.23\% \pm 1.51\%$) that was significantly higher than that from the Micromat II ($6.91\% \pm 1.34\%$) ($p = 0.014$) (see Table). All Micromat rapid test specimens from this site were capillary blood, obtained by finger-stick.
Similarly, the Micromat II correlated well with the boronate-affinity HPLC (n=74, Pearson r = 0.788, p < 0.001). Once again, the mean HbA1c results from the HPLC was significantly greater than the mean from the Micromat (7.75% ± 1.95% vs. 6.54% ± 1.96%, p < 0.001). The range of results from these two methods was 3.6% to 15.80%.

At this site, all of the Micromat specimens were not from capillary blood: 23% were from the venous blood apparatus, and 9% were from an EDTA blood sample. There was no significant difference (p = 0.23) in the means of samples collected by fingerstick and other methods.

Regarding feasibility and acceptability of introducing the same-visit Micromatt II test into the busy clinical practice setting, we found that medical assistants were able to collect and analyze samples and produce same-visit results. However, the five minute time dedication for each individual analysis was not well tolerated by staff because of numerous competing demands that made it difficult to perform all the test steps in the time intervals prescribed.

Discussion

Physicians in ambulatory settings routinely send blood samples to laboratories for HbA1c testing, often by HPLC analysis, and then wait several days for the HbA1c test results. Thus, patient counseling and treatment adjustments based on HbA1c levels are delayed, and at times follow-up can be lost completely.

Recent advancements in technology now make it possible for physicians to incorporate point-of-care HbA1c results to evaluate and adjust treatment of their diabetic patients. Studies of the effect of rapid HbA1c measurement found significantly improved glycemic control through 12-month follow-up [10, 11]. This technology is gaining
acceptance, and is now offered by a number of manufacturers. The same-visit HbA1c test provides the opportunity to improve diabetes care by discussing the value and adjusting management as needed during the same visit, rather than waiting until the patient can be telephoned and/or scheduled for a future visit. HbA1c testing has been studied for its effect on improved glycemic control in trials primarily conducted in specialty clinics. Yet, little is published regarding the validity of the same-visit test result, and the feasibility of using a rapid methodology in a busy primary care setting.

The manufacturer reports a correlation coefficient of 0.98 between the Bio-Rad Micromatt II and HPLC methodology. However, the correlation coefficients we obtained in this clinical situation (r = 0.713; and r = 0.788 for the two different HPLC methods) were significantly less than reported by the company. In addition, the mean HbA1c level was lower than that yielded from two types of HPLC analysis, and this difference spanned the treatment threshold level currently recommended by the American Diabetes Association (ADA) [12]. Thus, for some patients, the Micromatt II rapid test yielded a test result that was below the ADA treatment threshold of 7%, while the HPLC analysis produced a test result above 7%, suggesting the need for more intensive therapy.

Limitations

There are likely limitations to the generalizability of the study findings. First, the number of medical staff (n = 9) that collected samples and performed the HbA1c testing may have increased the variability of the same visit test results. Similarly, the correlation between the HPLC and the same visit methodologies may be improved when conducted under ideal conditions where sources of variation in the operation of the Micromat II are minimized. Secondly, we noted that introducing a research methodology into a busy
clinical practice setting is often met with a varying degree of resistance. Thus, evaluating
the acceptance of what may have been viewed by staff as a research technique may have
limitations when generalizing the acceptance of a clinical procedure. However, our
purpose was to conduct a correlation study in the real world setting of the busy FPC. We
trained all clinical staff in the calibration and specimen analysis of the point-of-care
instrument. From discussions with the clinical staff and physicians, we learned that there
was variability among staff members to faithfully adhere to the Micromatt II timed steps
as outlined in the test kit instructions.

Conclusions

Rapid HbA1c testing offers potential benefits for diabetes care, as patient tests are
available in the same visit. However, clinicians should be aware that the rapid HbA1c
technology may produce results slightly different than the method that they have been
utilizing, and that the same-visit test may suggest a different treatment strategy than a test
result from HPLC analysis. To overcome this barrier, we suggest that clinicians
determine how the results of an instant HbA1c test compare with the outside laboratory
reports on which they routinely base their treatment plans before incorporating the same-
visit HbA1c test into their practice.
Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: KLS conceived of the study, participated in the design of the study and helped to draft the manuscript. JCM participated in its design and coordination, analyzed the data, and helped to draft the manuscript. PAW participated in study design and coordination, and helped to draft the manuscript. AVN participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.
References


**Table.** Comparison of mean % HbA1c (SD) of paired samples using two HPLC methodologies and Bio-Rad Micromatt II assay.

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<thead>
<tr>
<th>HPLC Methodology</th>
<th>HPLC</th>
<th>MicroMatt II</th>
<th>p-value</th>
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<tr>
<td>Ion-exchange HPLC (n=73)</td>
<td>7.23 (1.51)</td>
<td>6.91 (1.34)</td>
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<td>Boronate affinity HPLC (n=74)</td>
<td>7.75 (1.95)</td>
<td>6.54 (1.96)</td>
<td>&lt;0.001</td>
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