Dried Blood Spot Measurement of Glycosylated Hemoglobin (HbA1c) in Wave I of the National Social Life Health & Aging Project

Authors:

Ada Gomero, University of Chicago, Department of Ob/Gyn

Thomas McDade, Northwestern University, Departments of Anthropology and Cells to Society (C2S)

Sharon Williams, Purdue University, Department of Anthropology

Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics, Chicago Core on Biomeasures in Population-Based Health and Aging Research at NORC and the University of Chicago Center on Demography and Economics of Aging *

* Corresponding author.
E-mail address: slindau@uchicago.edu (S.T. Lindau).
Fax: +1 773 834 5664


URL:

Date: October 8, 2008

Contents

- Rationale
- Measurement
- Population Norms
- Specimen Collection
- Storage and Shipping
- Assay
- Performance Characteristics
- Quality Control
Rationale

HbA1c, or glycosylated hemoglobin, is formed through the non-enzymatic binding of circulating glucose to hemoglobin (glycation). HbA1c is measured as the ratio of glycosylated to nonglycosylated hemoglobin (Peterson, Pavlovich et al. 1998). Higher levels of glucose in the blood contribute to more binding and consequent higher levels of glycosylated hemoglobin.

Glycation occurs over the entire 90-120 day life span of the red blood cell (Kilpatrick 2000). HbA1c can consequently be interpreted as an average of the blood glucose present over the past 3-4 months (Peterson, Pavlovich et al. 1998). Measurement of HbA1c is accepted as a useful index of mean blood glucose in the treatment of patients with diabetes (Rohlfing, Little et al. 2000). Decisions regarding treatment are often based on HbA1c. Although the American Diabetes Association (ADA) does not currently recommend HbA1c measurement for the diagnosis of diabetes, studies have shown frequency distributions similar to those of fasting plasma glucose (FPG) used in diagnosing diabetes (McCance, Hanson et al. 1994; Engelgau, Thompson et al. 1997; 2003). HbA1c is a more comprehensive measure of total glycemic exposure than FPG due to the representation of blood glucose in the postprandial state in addition to the fasting state (Rohlfing, Little et al. 2000).

HbA1c concentration is associated with diabetic microvascular complications (Rohlfing, Little et al. 2000; 2003), macrovascular complications (Khaw, Wareham et al. 2001; 2003), risk of death (Khaw, Wareham et al. 2001), and cardiovascular disease (Khaw, Wareham et al. 2001). Positive correlations with metabolic syndrome are also suggested (Grant, Soriano et al. 2004).

HbA1c <6% is considered normal (2007). The ADA-recommended treatment goal for the diabetic population is HbA1c <7% (2003), although the target may be higher for older adults (2007; Chin, Drum et al. 2008). HbA1c levels as low as 6.2% have been cited as at risk threshold values for development of cardiovascular complications (Rohlfing, Wiedmeyer et al. 2002; Grant, Soriano et al. 2004). Clinical and population studies have found racial, ethnic, and age disparities in HbA1c levels (Hashimoto, Futamura et al. 1995; Gilliland, Carter et al. 2002; Karter, Ferrara et al. 2002; Saaddine, Fagot-Campagna et al. 2002; Kirk, D'Agostino et al. 2006; Kirk, Passmore et al. 2008).

Measurement

Measurement of HbA1c is usually determined using whole blood samples via venipuncture. In NSHAP, blood was collected by finger-stick using a disposable lancet, and then captured on filter paper for transport and storage. Blood spot collection offers a
convenient, minimally invasive alternative to venipuncture (Lindau and McDade 2007; McDade, Williams et al. 2007). Blood spots facilitate in-home sample collection and can be performed by non-medically trained personnel (Williams and McDade 2008). Assay techniques for HbA1c include affinity chromatography, electrophoresis, high performance liquid chromatography (HPLC), and immunoassays. The “gold standard” of these methods is assay by HPLC (Halwachs-Baumann, Katzensteiner et al. 1997; Ray and Kerestan 2000). For the HbA1c assay used, high correlation of results is reported when compared to clinical standard methods (Flexsite; Williams and McDade 2008).

Interpretation of results from identical HbA1c samples assayed in different laboratories often varies due to absence of comprehensive HbA1c assay result standardization. The National Glycohemoglobin Standardization Program (NGSP) continues to standardize assay results to generalize them to larger studies and populations (Rohlfing, Little et al. 2000). The Roche Unimate method used by the NSHAP study is NGSP-certified.

**Population Norms**

Table 1 shows an age-dependent increase in HbA1c (Hashimoto, Futamura et al. 1995).

Table 1. Distribution of Mean HbA1c levels (% of total hemoglobin)

<table>
<thead>
<tr>
<th>Age</th>
<th>Men</th>
<th>Women</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-24 years</td>
<td>5.02%</td>
<td>4.95%</td>
<td>NHANES III; (Saaddine, Fagot-Campagna et al. 2002)</td>
</tr>
<tr>
<td>40-45 years</td>
<td>5.02%</td>
<td>4.1%</td>
<td>The Telecom Study; (Simon, Senan et al. 1989)</td>
</tr>
<tr>
<td>≥ 60 years</td>
<td>5.05%</td>
<td>5.32%</td>
<td>The Telecom Study; (Simon, Senan et al. 1989)</td>
</tr>
</tbody>
</table>

Although the use of HbA1c to diagnose conditions is not fully standardized, it is a recommended and accepted measure for risk of disease. Prevalence of retinopathy, for instance, is observed to increase sharply at HbA1c levels at or above 6.2% (2003).
Table 2. NSHAP Summary Statistics for HbA1c levels (% of total hemoglobin) (Williams and McDade 2008)

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Range (% of total Hb)</th>
<th>Mean (weighted) HbA1c (% of total Hb)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ages 57-65</td>
<td>4.70-13.4</td>
<td>6.21</td>
<td>1.39</td>
</tr>
<tr>
<td>ages 66-75</td>
<td>4.60-11.4</td>
<td>6.07</td>
<td>0.89</td>
</tr>
<tr>
<td>ages 76+</td>
<td>4.20-14.2</td>
<td>6.09</td>
<td>1.02</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ages 57-65</td>
<td>4.50-12.5</td>
<td>5.99</td>
<td>0.99</td>
</tr>
<tr>
<td>ages 66-75</td>
<td>4.50-11.4</td>
<td>5.91</td>
<td>0.82</td>
</tr>
<tr>
<td>ages 76+</td>
<td>4.60-13.5</td>
<td>6.04</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**Specimen Collection**

The blood spot module was randomized to 5/6 of the total NSHAP sample (N=2494), of these, 84.5% (2,105) participated. There were no significant differences between those who agreed to provide blood spots and those who did not with respect to gender, race, ethnicity, age, education, income level, marital status, self-reported mental or physical health, or the reported number of doctor’s visits. Due to collection difficulties, blood spots were not collected from an additional 57 individuals who originally agreed to participate. In total, dried blood spots were collected from 2,048 individuals. From these, 1739 HbA1c analytes were obtained.

Blood was obtained from free-flowing capillary blood via a fingerstick with a retractable-tip disposable lancet. NSHAP acquired two lancets (model details in Product Availability section below). The Surgilance lancet was used routinely, contained a 2.3 mm blade and was slightly less penetrative. The BD lancet, which contains a slightly larger blade and is commonly used for newborn heel-sticks, was used for cases of thick calluses or where the fingerstick specimen was difficult to obtain with the Surgilance. The blood was allowed to pool on the respondents’ finger and four drops of blood were dropped onto filter paper, the first drop being reserved for future genetic analysis. Overall, the procedure required approximately 8 minutes.

**Interviewer instructions**

- Angle Respondent’s hand below their lap.
- Warm finger and stimulate circulation by gently kneading and squeezing the appropriate finger.
- Ask Respondent to gently shake their hand a few times.
- Wipe the index finger of the right hand with alcohol swab and wait a few seconds for the alcohol to dry (DO NOT blow on finger, wave hand, etc. to speed up drying).
- Squeeze the finger just below the area to be pricked.
- Firmly prick finger in the fleshy part of the pad, just off the center.
- IMMEDIATELY dispose of the lancet into the sharps container.
- Allow blood to well on tip of finger.
- If necessary, apply gentle pressure below the site of the prick
- Place first drop in discard circle of filter paper – marked D
- Place 3 (if possible) additional drops on filter paper.
- If unable to fill 3 spots (+ discard spot): Prick another finger. Place first drop in discard circle of filter paper (marked D) and place additional drop(s) in remaining circles.
- If necessary: Ask Respondent to hold cotton ball on finger and apply pressure until bleeding stops.
- Offer Respondent a bandage.
- Label filter paper with su_id.
- Fill out blood spot collection form.
- Place filter paper in plastic bag with desiccant and seal.
- Store at 4°C until shipment.

The filter paper was allowed to dry for the remainder of the interview before being placed in a plastic bag with desiccant for transportation.

Storage and Shipping

After the interview, the filter paper was placed in a Ziploc bag. Once the field investigator reached home, the filter paper was removed from the Ziploc bag and placed in a clear plastic container with the filter paper cover flipped up. A desiccant pack was placed in the container which was then sealed overnight and left at room temperature to ensure drying of the blood spots. In the morning, the filter paper was stored in a sealed Ziploc bag along with the desiccant pack and placed in a storage container. The storage container was kept in the refrigerator at 4°C until shipping day. Blood spots were shipped at room temperature to the designated location. Upon arrival at the laboratory, the filter papers were catalogued and frozen at -25°C until analysis.

| Shipping Address | Thomas McDade, PhD  
Northwestern University  
Associate Professor  
Department of Anthropology  
Laboratory for Human Biology Research  
1810 Hinman Ave.  
Evanston, IL 60208 |
|-------------------|---------------------------------------------------|

Blood spots to be assayed for HbA1c were then sent to a central analytical laboratory.

| Laboratory Shipping Address | Flexsite Diagnostics, Inc.  
3541 SW Corporate Pkwy  
Palm City, FL 34990  
Contact: Robert Ray 1 772 221 8893 |
|----------------------------|-------------------------------|

Assay
NSHAP used blood spot filter paper collection of capillary blood for assay of HbA1c. One spot on the filter paper from Flexsite Diagnostics was pretreated with a proprietary solution to facilitate re-hydration of the dried blood prior to analysis. At the laboratory, blood was eluted from the blood spot on the filter paper. The Roche Unimate immunoassay and Cobas Integra Analyzer were used to perform the assay. The immunoassay incorporates a latex-enhanced competitive turbidimetric immunoassay, which determines HbA1c concentration, with a colorimetric quantification of total hemoglobin. The Cobas Integra analyzer, which is NGSP-certified, is calibrated using a synthetic HbA1c standard (Ray and Kerestan 2000).

Performance Characteristics

A. Precision

Precision for this assay was determined by assaying two normal blood spots and two abnormal (elevated) blood spots twice daily for twenty days, following the National Committee for Clinical Laboratory Standards procedure EP-5A (Ray and Kerestan 2000). The American Diabetes Association recommends a CV < 5%.

Table 3. Typical precision for HbA1c assay with normal and abnormal blood spots on treated filter paper (stored at -70ºC)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Run CV</td>
<td>0.88%</td>
<td>1.71%</td>
</tr>
<tr>
<td>Between Run CV</td>
<td>1.31%</td>
<td>1.90%</td>
</tr>
<tr>
<td>Total (20 day) CV</td>
<td>1.46%</td>
<td>2.54%</td>
</tr>
</tbody>
</table>

Reproduced with permission from Robert Ray, Flexsite Diagnostics

B. Accuracy

Accuracy was determined by comparing physician-collected dried blood spot samples to venipuncture whole blood hemolysate samples. Correlation of the blood spot HbA1c obtained from the doctor’s office blood specimen with the sample obtained from the venipuncture specimen was r=0.978 (Ray and Kerestan 2000).

C. Correlation with Serum

The correlation of home-collected blood spots (from same patients) with whole blood was r=0.956. Assay results are highly correlated with the standard Roche Unimate immunoassay on whole blood (Ray and Kerestan 2000).

D. Comparison with reference technology Diamat HPLC

Correlation of this method with HPLC (r=0.981) indicates that results are highly accurate relative to the “gold standard” method (Ray and Kerestan 2000).
Quality Control

Blood spots were obtained on a card containing five pre-printed circles of standard size (1/2 inch/12.7mm diameter). Excellent samples filled the entire circle. Good samples filled a large (6.0mm) hole-punch. Poor samples were ones that did not fill a small (3.2mm) hole punch. Cards were adequate if they allowed for one large and three small punches and excellent if they allowed for more. Throughout the study, the number, quality and condition of the blood spots was recorded by personnel, and if consistent problems were observed from a single field interviewer, they were contacted to discuss problems and techniques to improve the quality of blood spot collection. (Williams and McDade, 2007).

The stability of HbA1c in dried blood spots for this assay under typical mailing conditions was assessed by comparing blood spots on filter paper to whole blood hemolysate values at room temperature at 1, 7, and 14 days from collection. Correlation coefficients at each time period were $r > 0.960$, demonstrating high stability of the blood spot analyte at room temperature. A minimal slow rate of glycation was observed in the dry state samples over two weeks. Only minimal losses of glycation, which do not detract from the value of this technique, were observed in assessments of different mailing conditions. Further, no significant effect on the samples exists from multiple cycles of freezing and thawing, demonstrating the excellent stability of dried blood spots (Ray and Kerestan 2000).

Scoring & Usage

The NSHAP dried blood spots sample size for HbA1c was 1739. Results are reported as percent of glycosylated hemoglobin (of total hemoglobin) (Williams and McDade 2008).

Product Availability

<table>
<thead>
<tr>
<th>Product Name</th>
<th>A1c At-Home</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Flexsite Diagnostics, Inc.</td>
</tr>
<tr>
<td>Location of Manufacturer</td>
<td>Palm City, FL</td>
</tr>
<tr>
<td>Contact: Robert Ray 1 772 221 8893</td>
<td></td>
</tr>
<tr>
<td>Catalogue No.</td>
<td>Custom Product</td>
</tr>
<tr>
<td>Product Name</td>
<td>903™ Multiple-part Neonatal Card</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Schleicher &amp; Schuell BioScience</td>
</tr>
<tr>
<td>Location of Manufacturer</td>
<td>Keene, NH  03431</td>
</tr>
<tr>
<td>Contact: Judy Peter 800- 437-7003</td>
<td></td>
</tr>
<tr>
<td>Catalogue No.</td>
<td>10 537 279</td>
</tr>
<tr>
<td>Product Name</td>
<td>SurgiLance Safety Lancet, 2.3 mm (blue)</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>SurgiLance, Inc.</td>
</tr>
<tr>
<td>Location of Manufacturer</td>
<td>Norcross, GA</td>
</tr>
<tr>
<td>Tel: +1 (770) 448 9493 (US)</td>
<td></td>
</tr>
<tr>
<td>Fax: + 1 (877) 804 5240 (Toll Free) (US)</td>
<td></td>
</tr>
<tr>
<td>Catalogue No.</td>
<td>SLB250</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Product Name</td>
<td>BD Quikheel™ Preemie Lancet (pink)</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Becton, Dickinson and Company</td>
</tr>
<tr>
<td>Location of Manufacturer</td>
<td>Franklin Lakes, NJ</td>
</tr>
<tr>
<td>Catalogue No.</td>
<td>368100</td>
</tr>
</tbody>
</table>

**Sources**

Procedure developed December 2004 from recommendations by Stacy T. Lindau, University of Chicago, Thomas McDade, Northwestern University and Sharon Williams, Northwestern University. Assay developed by Thomas McDade at Northwestern University Laboratory for Human Biology Research (currently unpublished).

**Acknowledgement**

The authors would like to thank Robert Ray of Flexsite Diagnostics for reviewing and contributing to this report.

**References**


