THE HEALTH AND RETIREMENT STUDY:
INVESTIGATION OF DRIED BLOOD SPOT HBA1C
MEASUREMENT ERRORS USING TIPTEMPT STAMPS

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HBA1C FROM DBS

- Used clinically, commercial laboratories have developed assays for A1c that can be done using dried blood spots (DBS)
- Several correlation studies have shown that HbA1c results obtained from DBS are a reliable alternative to whole blood
- DBS can be self-administered by respondent or IWER-assisted
- For large population-based surveys this has meant that HbA1c measurement is possible even when transporting whole blood is not feasible
HEMOGLOBIN A1C (HBA1C)

- Blood-based biomarker that summarizes the average levels of blood glucose over a two- or three-month period (avg life of a red blood cell)
- Does not require fasting and can be done from blood collected at any time of the day
- In contrast, glucose levels vary widely over the course of a day and in response to the intake of food, making standard point-in-time readings very difficult to interpret in isolation
PREVIOUS STABILITY STUDIES

• Dried blood spots can be stored at room temperature for up to 1 month or in a freezer for up to three months and HbA1c values will remain stable (Buxton 2009)
HB MEASUREMENT ERRORS

- Data from the past 2 waves of the Health and Retirement Study show an increase in HbA1c error rates during the summer months of data collection due to a possible “false” hemoglobin variant leading us to reexamine the effect of temperature on HbA1c stability
PERCENT OF RESPONDENTS WITH HBA1C MEASUREMENT ERROR BY COLLECTION MONTH - 2014
HB VARIANTS – BOTH REAL AND FALSE

- Hemoglobin variants: mutated forms of hemoglobin that may affect the distribution of HbA1c scores
- HbC, HbE, HbD, HbS – most common
- Real Hb variants present in 2% of the HRS sample
- More common in African Americans
- The accuracy of HbA1c levels is negatively affected by the presence of hemoglobin variants (Little 2009)
- Method specific – not all assay methods are affected to the same extent
HB VARIANTS – BOTH REAL AND FALSE

• False variants present as samples with low HbS – the amount of the variant as a percentage of total hemoglobin is not clinically plausible

• Erroneous identification of HbS affects the calculation of %HbA1c value (too high)

• Could be a degradation problem

• Highly reproducible

• Collection protocol may be causing systematic error in HbA1c scores
Example of a whole blood sample analyzed on the Bio-Rad Variant II Hemoglobin Testing System (HPLC)

HbA1c peak is identified at \( \approx 0.95 \text{min} \) →

HbA peak is identified at \( \approx 1.75 \text{min} \) →

Complex algorithm integrates curve under A1c peak (i.e., the shaded area at 0.95min)

\[ \% \text{HbA1c} = \frac{\text{HbA1c area (shaded)}}{\text{HbA area}} \]
normal %HbA1c QC sample

5.4% HbA1c

high %HbA1c QC sample

10.2% HbA1c
DBS study samples: HbS Variant (A & B) vs. False HbS variant (C & D)

A1c Concentration = 8.9%  
HbA1c = 6.9%

**Total Hb = 38% HbS  
HbA1c = 6.9%**

False HbS added back to HbA

**Total Hb = 11% false HbS  
HbA1c = 6.1% = actual HbA1c = 5.5%**

False HbS
HB VARIANTS – MONITORING TEMPERATURE

• During the 2014 field period, approximately 3000 DBS cards were tagged with temperature stamps capable of measuring temperature up to 130F.

• The temperature the DBS card has been exposed to was recorded both at the time of blood collection and upon arrival at the lab.
TIP TEMP

• Non-reversible temperature labels that indicate thermal reports (0, 110, 120, 130 degrees)
• Temperature stamps from the cards show the highest temperature the card is exposed to during travel
• Approx. $1 each
• Customizable
• Tip Temperature Products
  www.tiptemp.com
HBA1C ASSAY

• HbA1c assay for 2014 sample conducted at the University of Washington Department of Laboratory Medicine using automated ion-exchange high-performance liquid chromatography (HPLC)
• Well-suited to identifying aberrant peaks produced by variants
COLLECTED VARIABLES

- Age of respondent
- Race/ethnicity
- Temperature pre collection
- Temperature post collection
- Time to arrival at lab
- Evidence of drying errors
- Month of collection
- Region
- IWER effects
RESULTS

• 7300 DBS cards – 13% (n=957) possible Hb variant error
• 2743 cards with Tip Temp Stamps (June-Nov)
• 16% all cards experienced post temp of >110 F
• 3% experienced post temp of > 130F
• Mean days to lab cards with no error = 4.41 days
• Mean days to lab cards with Hb error= 6.34 days
## RESULTS

<table>
<thead>
<tr>
<th><strong>Factor</strong></th>
<th><strong>OR</strong></th>
<th><strong>95% Wald Confidence Limits</strong></th>
<th><strong>Pr &gt; ChiSq</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.995</td>
<td>0.986 - 1.004</td>
<td>0.2743</td>
</tr>
<tr>
<td>Black</td>
<td>0.796</td>
<td>0.605 - 1.048</td>
<td>0.1042</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.285</td>
<td>1.027 - 1.607</td>
<td>0.0282</td>
</tr>
<tr>
<td>Post Temp 110</td>
<td>2.006</td>
<td>1.5 - 2.682</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Post Temp 120</td>
<td>0.644</td>
<td>0.386 - 1.075</td>
<td>0.0924</td>
</tr>
<tr>
<td>Post Temp 130</td>
<td>2.234</td>
<td>1.175 - 4.248</td>
<td>0.0143</td>
</tr>
<tr>
<td>Drying Errors</td>
<td>0.819</td>
<td>0.533 - 1.257</td>
<td>0.3604</td>
</tr>
<tr>
<td>Days to Lab</td>
<td>1.103</td>
<td>1.071 - 1.136</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Summer Month</td>
<td>1.708</td>
<td>1.365 - 2.137</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
SUMMARY

• Temperature exposure is likely a contributing factor in HbA1c measurement error
• No evidence of pre collection temperature, region, or IWER, drying/ collection error effects
• Not all cards with an error were exposed to high temperatures, so there must be another factor contributing to false Hb variant measurement error
LAB REPLICATION

• Exposed dried DBS to various times & temperatures
  • Some %HbA1c values dropped
  • Did not cause low HbS to appear

• Exposed wet DBS to various times & temperatures
  • Blood welded to the filter paper - no results
  • Few samples that did elute did not have low HbS
FUTURE RECOMMENDATIONS

- Results aren’t strong enough to significantly change the protocol ($$), but interviewers will be instructed to handle DBS cards more carefully especially in areas or months with high temperatures
- Reinforce prompt shipping protocol
- Electronic recalculation of %HbA1c after identification of false variants may be a good strategy – requires using HPLC
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