

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

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HRS | HEALTH AND
RETIREMENT
STUDY

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

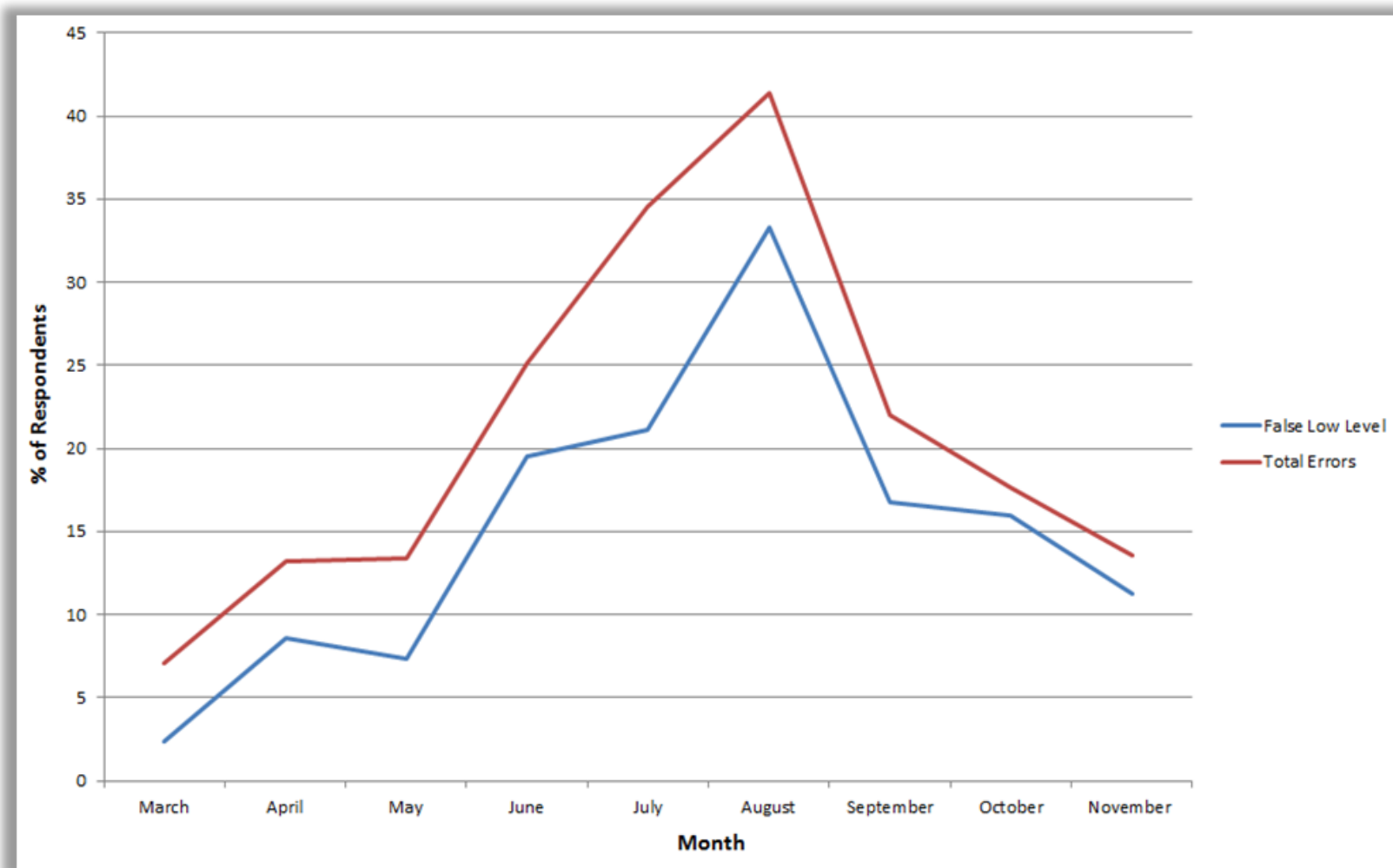
PREVIOUS STABILITY STUDIES

- 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)

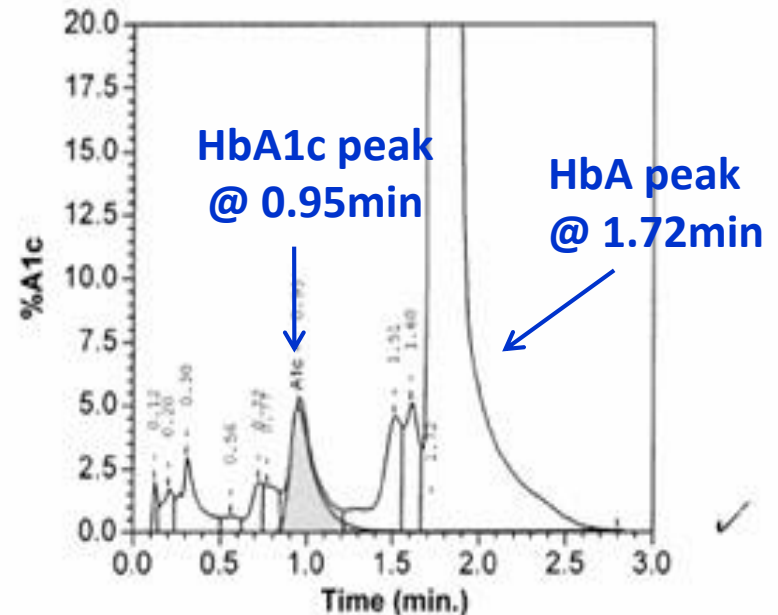
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.3	0.12	6419
A1a	---	0.5	0.20	12258
A1b	---	1.3	0.30	32780
F	---	0.3	0.56	6824
Unknown	---	0.6	0.72	16145
LA1c	---	0.7	0.77	16390
A1c	5.0	---	0.95	79815
P3	---	2.6	1.51	63954
P4	---	1.8	1.60	45105
Ao	---	88.8	1.72	2212468

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

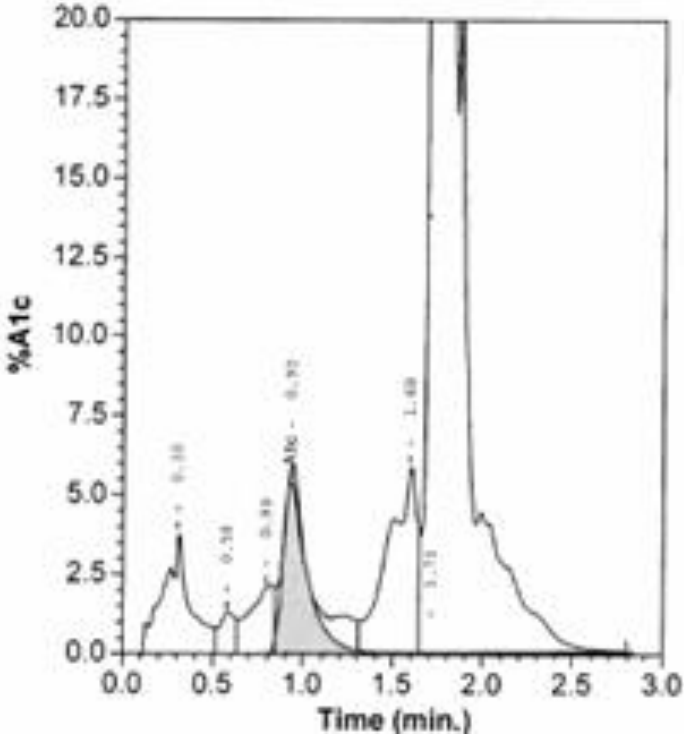
HbA peak is identified at $\approx 1.75\text{min}$ →

A1c Concentration = 5.0 % Total Area: 2,492,157 ✓

Complex algorithm integrates curve under A1c peak (i.e., the shaded area at 0.95min)
 $\% \text{HbA1c} = \text{HbA1c area (shaded)} \div \text{HbA area}$

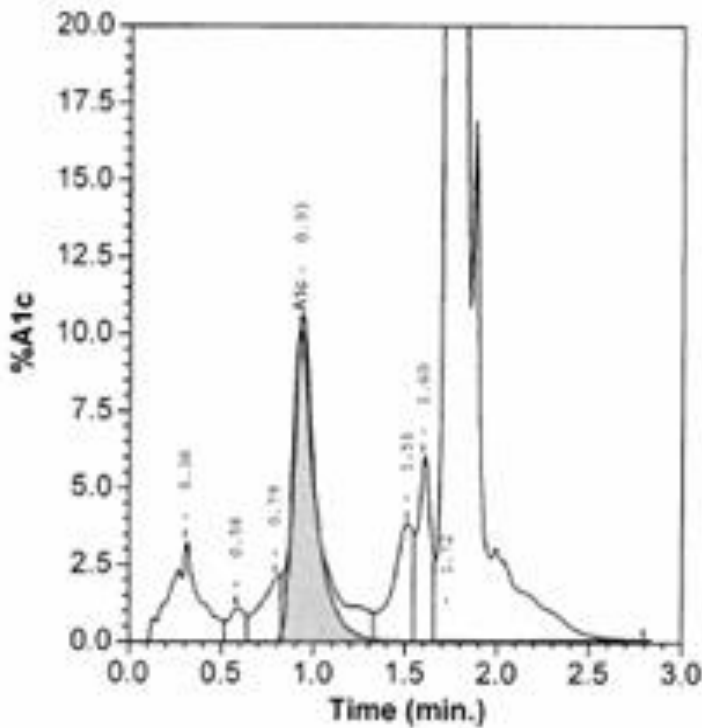


normal %HbA1c QC sample



5.4% HbA1c

high %HbA1c QC sample



10.2% HbA1c

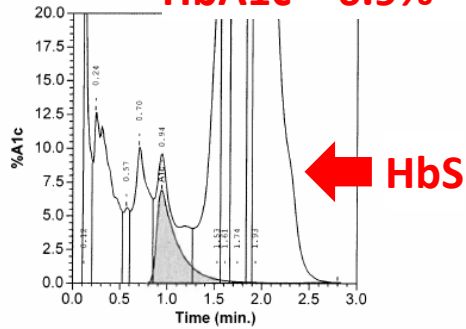
DBS study samples: HbS Variant (A & B) vs. & False HbS variant (C & D)

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
A1a	---	2.6	0.12	48016
A1b	---	4.5	0.24	83160
F	---	0.7	0.57	12509
LA1c	---	2.9	0.70	53432
A1c	6.9*	---	0.94	51881
P3	---	5.3	1.53	98603
P4	---	4.3	1.61	79601
Ac	---	39.0	1.74	724513
S	---	37.9	1.93	703644

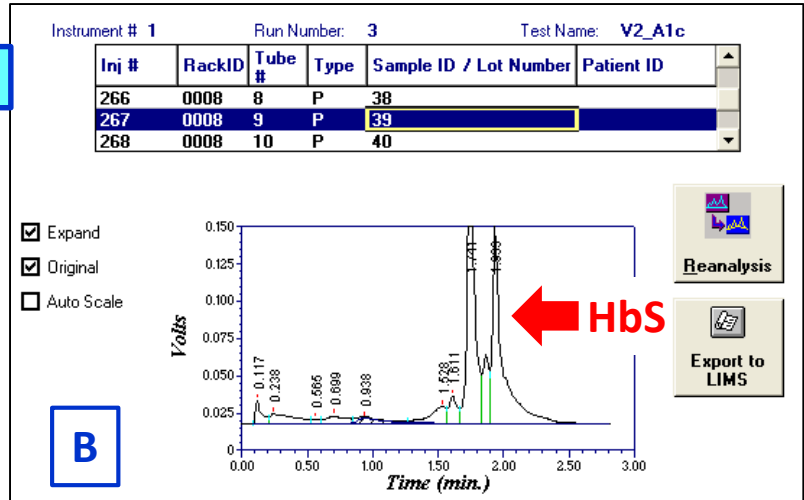
*Values outside of expected ranges

A1c Concentration = 6.9* %

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



A



B

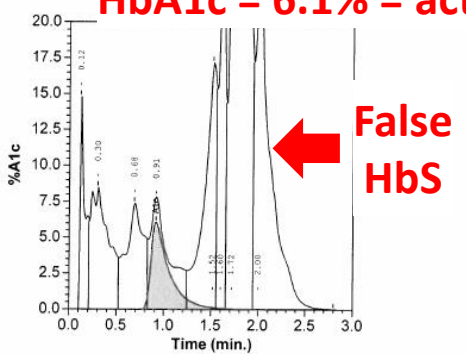
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
A1a	---	2.3	0.12	162379
A1b	---	4.9	0.30	1838684
LA1c	---	4.1	0.68	339562
A1c	6.1*	---	0.91	
P3	---	6.9	1.52	
P4	---	5.4	1.60	
Ac	---	61.3	1.72	
S	---	11.3	2.00	

*Values outside of expected ranges

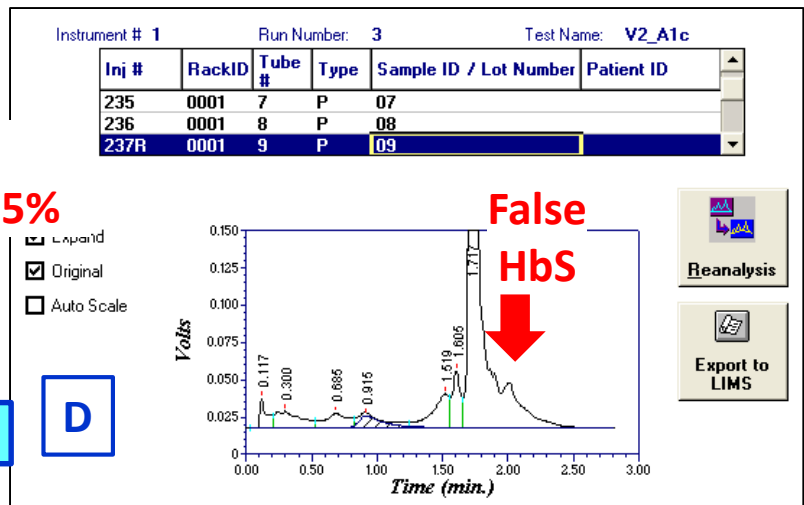
A1c Concentration = 6.1* %

**False HbS
added back
to HbA**

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



C



D

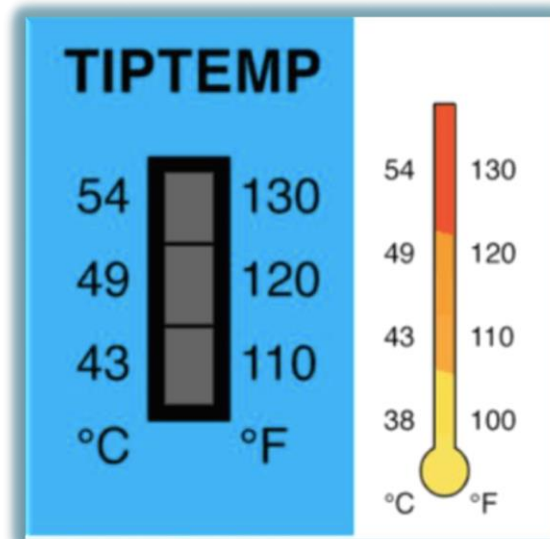
HB VARIANTS – MONITORING TEMPERATURE

- 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

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

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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REFS

Buxton, O., Malarick, K., Wang, W., & Seeman, T. (2009) Changes in dried blood spot HbA1c with varied post-collection. *Clin Chem*, 55(5)

Little, R., & Roberts, W. (2009) A Review of Variant Hemoglobins Interfering with Hemoglobin A1c Measurement. *Journal of Diabetes Science and Technology*, 3(3)