Changes in Dried Blood Spot Hb $A_1c$ with Varied Postcollection Conditions

To the Editor:

Diabetes affects $>5\%$ of US adults (15% of those older than 60 years) and is on the rise in adolescents. Glycosylated hemoglobin (HbA1c), a cumulative marker of blood glucose concentrations over the previous 2 months, has become a powerful clinical tool for diabetes management (2) and is predictive of future complications of diabetes (3). Large-scale community-based studies often preclude the option of phlebotomy and would benefit from simplified sample handling. Epidemiologic studies have begun to use Hb A1c in dried blood spots as a biomarker (5). We used Hb A1c, from dried blood spots by validating the storage conditions appropriate for large-scale epidemiologic/outreach studies to test the hypothesis that measurements of Hb A1c in dried blood spots are valid under low-intensity storage conditions.

All participants were studied at the Brigham and Women’s Hospital, Boston, Massachusetts; all procedures were conducted in accordance with the Declaration of Helsinki.

Blood samples were drawn via standard phlebotomy procedures into EDTA-containing tubes for duplicate HPLC analyses of Hb A1c (Tosoh G7 automated HPLC assay, Brigham and Women’s Hospital Hematology Laboratory). Intrainrun imprecisions (CVs) were 0.5% and 0.9% at Hb A1c proportions of the total hemoglobin of 0.041 and 0.1335, respectively. Interrun CVs were 1.6% for control samples with Hb A1c values of 0.064 and 0.7% for control samples with Hb A1c values of 0.11. Blood for spotting was drawn into an identical EDTA tube, and a syringe was used to let each drop of blood fall onto randomly assigned blood-spot cards (3 spots/card) within 1 min. Inversion of the syringe minimized the settling of red blood cells. Samples were air-dried for at least 20 min and then placed into single-sample, airtight bags that included a desiccant pouch. Trios of blood-spot cards were processed identically. After storing the samples at room temperature for 0, 2, or 4 weeks, we shipped the samples to Biosafe Laboratories for Hb A1c analysis (conditions 1–3) or placed them in freezer storage ($\sim80 ^\circ C$) for an additional 4 or 12 weeks (conditions 4–6, 7–9). Sample trios were shipped by US mail, presumably at room temperature, in batches that included multiple individuals/conditions per pack. Hb A1c measurements of dried blood spots were performed blinded to storage conditions and protocol. We used Roche Hemolyzing Reagent to elute from 3.00-mm punches of homogeneous parts of the blood spot and analyzed the eluate with the Roche Modular system with Roche Hb A1c reagents, an immunoturbidimetric assay with results that show good correlation with the standard Hb A1c methodology for whole blood [$r^2 = 0.9708$; $y = 0.85x + 0.81$ (n = 115)]. The Roche assay exhibited intrainrun CVs of 2.1% at Hb A1c values of 0.05 and 1.4% at Hb A1c values of 0.076. Interrun CVs were 4.1% and 3.5% for the same Hb A1c values. We applied mixed-effects ANOVA models to compare the effects of different storage conditions and times on Hb A1c values measured from dried blood spots and measured by the HPLC assay. We repeated the analysis with dried blood spots that had undergone minimal storage. To identify conditions that degraded samples, we compared SD and bias measures for the trio (3 spots/card).

Twelve individuals [4 diabetic and 8 nondiabetic individuals; mean (SD) age, 46.8 (12) years; mean body mass index, 31 (9) kg/m$^2$] completed the protocol. The SD of trios was $<0.0012$ (i.e., proportion of glycated hemoglobin) across all storage conditions and times; differences between the times of storage at room temperature [$F(2,88) = 1.44; P = 0.242$], of storage in freezers [$F(2,88) = 1.90; P = 0.156$], or their interaction [$F(4,88) = 1.41; P = 0.236$] were not statistically significant. The mean bias of the assays of dried blood spots compared with the gold standard HPLC assay was $-0.00099$ (i.e., proportion glycosylated hemoglobin) (range, 0.0044–0.00300). The bias was greater at higher Hb A1c values (Fig. 1). This bias is expected because of systematic differences in the immunoturbidimetric and HPLC assay methods described above. Bias did not vary with the Hb A1c value when the referent condition was dried blood spots with minimal storage handling (data not shown).

The main effect of the duration of room temperature storage was significant [$F(2,304) = 5.26; P = 0.006$], but the main effect of the freezer duration was not [$F(2,304) = 0.76; P = 0.467$]. Interactions between room-temperature and freezer-storage durations were significant [$F(4,304) = 10.56; P < 0.0001$] but not in a systematic manner, suggesting that the number of storage-condition transitions affects bias.

We determined that simplified sample handling (without refrigeration or freezing) could be a valid means for obtaining reliable Hb A1c estimates. Storage at room temperature for up to a month or freezer storage for up to 3 months after collection yielded stable Hb A1c values. Across a range of stor-
age conditions, we observed bias of approximately $-0.001$ (proportion glycosylated hemoglobin) relative to results obtained with the gold standard HPLC method. We conclude, given the limitations of a small sample size and design that did not examine all permutations of storage conditions, that storage at room temperature is adequate; however, we encourage investigators to process and assay their samples as rapidly as practicable. The data obtained in this validation of methods and conditions appropriate for large-scale community and outreach studies extends the usefulness of Hb $A_1c$ as a marker.

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References


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