Evaluation of Capillary Collection System for HbA_{1c} Specimens

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OBJECTIVE— We evaluated the accuracy and stability of a capillary HbA_{1c} collection system for use with a high-performance liquid chromatography analyzer.

RESEARCH DESIGN AND METHODS— The collection system requires that 5 ul blood is drawn into a calibrated capillary tube, which is then placed into a vial of stabilizing solution and sent for analysis. The study was conducted on simultaneously drawn capillary and venous blood specimens from 47 pediatric diabetes patients. Accuracy was determined by comparing the capillary to the venous HbA_{1c} values. Stability was measured by analyzing 17 capillary specimens over 3 wk.

RESULTS— There was excellent agreement between the capillary and venous HbA_{1c} values (capillary 0.959, venous +0.494, R^2 = 98.7%). The capillary HbA_{1c} values were 0.2% higher than the venous HbA_{1c} values and decreased gradually over time (0.1% HbA_{1c}/week) when stored at room temperature.

CONCLUSIONS— The Bio-Rad (Richmond, CA) collection system is accurate, stable, and simple to use.

The measurement of HbA_{1c} is a well-accepted indicator of long-term glycemic control and is very important in the management of insulin-dependent diabetes. Although the use of capillary specimens for whole-blood glucose analysis is standard, most laboratories collect venous specimens for HbA_{1c} testing. Although children with diabetes become accustomed to capillary blood draws, many develop an aversion to venipuncture. The psychological trauma of venipuncture can be so severe that some parents and children do not permit venous specimens to be obtained. This deprives the physician of the ability to monitor the patient's long-term glycemic control and can have a detrimental effect on the treatment of diabetes. To address this problem in our pediatric diabetes clinic, we were asked to investigate capillary blood collection systems for HbA_{1c}.

Several authors have described methods of capillary collection of specimens for HbA_{1c} (1–4). Although these methods have demonstrated good correlation to venous HbA_{1c} values, they generally require a larger blood sample and longer, more complex sample preparation. The methods used to evaluate these collection systems included colorimetric (1,4), affinity chromatography (2), and isoelectric focusing (3). Although Slemenda et al. (5) evaluated a reproducible filter paper capillary collection system, the correlation to the reference laboratory results was poor.

RESEARCH DESIGN AND METHODS

— The Bio-Rad Diamat high-performance liquid chromatography (HPLC) analyzer was used to analyze the HbA_{1c} specimens. The Diamat uses a cation-exchange resin and graduated phosphate buffers to separate and quantitate the various Hb fractions. The Bio-Rad HbA_{1c} capillary collection system (6) consists of a heparinized glass capillary tube, a holding device, and a plastic vial containing 1 ml stabilizing solution of EDTA and KCl. The capillary tube (calibrated to hold 5 ul when filled from end to end) is filled with blood and is then put into the stabilizing solution and shaken gently until all of the blood is in solution. The tube can remain in the vial throughout the analyses. No further treatment is required for specimens >24 h old. Analysis of specimens within 24 h of collection requires addition of 100 ul boric acid–developing reagent and incubation at 37°C for 30 min to complete removal of the labile fraction.

Capillary specimens and one 5 ml EDTA venous specimen were obtained from 47 pediatric diabetes patients at Park Nicollet Medical Center. The venous specimens were refrigerated and assayed within 2 days of collection. The venous HbA_{1c} values were used as

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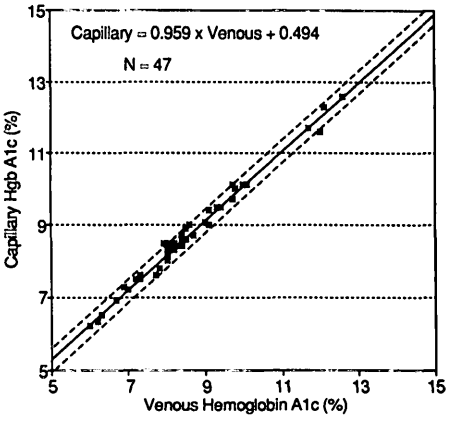

Figure 1—*Regression of capillary vs. venous HbA_{1c}. Dashed lines, 95% confidence intervals.*

the reference. Two capillary specimens were drawn from 30 patients. The first capillary specimen was assayed on day 1 and the second specimen on day 2. A single capillary puncture was used to obtain five capillary specimens from each of the other 17 patients. One capillary specimen was assayed on day 1, and the rest of the stabilized capillary specimens were assayed between 2 and 22 days after collection to determine stability of specimens over time. All capillary specimens were stored at room temperature until assayed. Linear regression and paired *t* test analyses were used to evaluate the agreement between the venous and capillary values.

RESULTS — The reference range for our Diamat HPLC was 4.2–5.8, and the mean ± SD interassay variability of the Diamat during the study period was 5.02 ± 0.11 and 9.65 ± 0.15%. All chromatographs were normal in appearance. The HbA_{1c} values ranged from 6 to 12.6% and 6.2 to 12.6% for the venous and capillary specimens, respectively. Figure 1 illustrates the agreement between the capillary and venous HbA_{1c} values. The equation of the regression line is capillary HbA_{1c} = 0.959 venous HbA_{1c} +0.494, R^2 = 98.7%, and SE of the estimate = 0.17%. For capillary specimens assayed within 3 days, the paired *t*-test analysis showed that the capillary HbA_{1c} values were significantly different from their venous counterparts ($P < 0.01$), with a mean difference of 0.2% (range −0.4–0.6%). There was no relationship between the paired differences and the magnitude of the venous HbA_{1c} concentration. Table 1 shows that the capillary/venous differences were time dependent, with the capillary values slowly decreasing over time (~0.1% HbA_{1c}/week) when the capillary specimens were stored at room temperature.

CONCLUSIONS — The Bio-Rad capillary HbA_{1c} collection system is a simple method for collecting and preserving capillary specimens and is specific to the Diamat HPLC analyzer. Capillary specimens correlate well to venous values, with the capillary values being slightly higher than their venous counterparts. The capillary collection can be performed by nursing staff or patients and requires minimal training. The specimens are very stable, making it feasible to ship or gather specimens from outlying areas. We have used the capillary collection system for ~1 yr in our pediatric diabetes clinic and an affiliate offsite collection program.

Table 1—*Change in capillary HbA_{1c} values over time when specimens are stored at room temperature. Bias is the difference between mean capillary HbA_{1c} and mean venous HbA_{1c} (n = 17)*

Day of assay	Mean capillary HbA_{1c} (%)	Bias (%)
1	9.0	+0.2
2–4	9.0	+0.2
5–9	8.9	+0.1
9–14	8.8	0.0
15–22	8.7	−0.1

After completion of the study, we assayed a series of samples that were collected off site with one lot number of kits and transported at ambient temperatures. Many of these specimens demonstrated degradation peaks. We believe that this was an isolated event, because no other samples have exhibited similar problems in ~1 yr of testing. We recommend that, if specimens are to be sent at ambient temperatures, further studies be done to establish the stability of these specimens during shipping. The Bio-Rad capillary HbA_{1c} collection system is accurate, stable, simple to use, and encourages the testing of patients who have an aversion to venipuncture.

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References

1. Little RR, Wiedmeyer HM, England JD, Knowler WC, Goldstein DE: Measurement of glycosylated whole blood protein for assessing glucose control in diabetes: collection and storage of capillary blood on filter paper. *Clin Chem* 31:213–16, 1985
2. Little RR, McKenzie EM, Wiedmeyer HM, England JD, Goldstein DE: Collection of blood on filter paper for measurement of glycated hemoglobin by affinity chromatography. *Clin Chem* 32:869–71, 1986
3. Larsen ML: Evaluation of a new capillary blood collection system for laboratory assay of glycated haemoglobin. *Scand J Clin Lab Invest* 46:315–17, 1986
4. Rendell M, Brannan C, Nierenberg J, Rasbold K, Hestorff T: Fingerstick glycosylated hemoglobin, plasma protein, and albumin. *Diabetes Care* 10:629–32, 1987
5. Slemenda CW, Marrero DG, Fineberg SE, Moore PS, Gibson R: Mail-in paper strip vs. microcolumn technique for measurement of glycosylated hemoglobin. *Diabetes Care* 13:886–88, 1990
6. Bio-Rad Laboratories: Diamat HbA_{1c} test sample preparation kit instruction manual. Hercules, CA, Bio-Rad, 1988