Mail-In Paper Strip vs. Microcolumn Technique for Measurement of Glycosylated Hemoglobin

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We compared glycosylated hemoglobin (GHb) determined from capillary blood samples on paper strips with a standard microcolumn technique in a cross-sectional observational study with laboratories blinded to duplicate samples. Both the standard and the filter strip laboratories were provided with 80 uniquely identified blood samples from 40 individuals. Each laboratory ran duplicate analyses on each sample, yielding 160 GHb values. The within-laboratory correlations between blinded duplicates were 0.98 for the standard (microcolumn technique) and 0.94 for the filter paper (affinity technique) laboratories. The between-laboratory correlations ranged from 0.69 to 0.77. When classifying patients by quartile of glycemic control, the laboratories agreed on 60% of the patients. In an effort to identify sources of between-laboratory variability, varying quantities of blood were applied to strips and reanalyzed. Five microliter drops always yielded inflated estimates of GHb. These data suggest that the estimates of GHb obtained from mail-in paper strips, although internally consistent, differ in important ways from standard laboratory values, reemphasizing the need for caution in the interpretation of interlaboratory and intermethod comparisons. *Diabetes Care* 13:886–88, 1990

Glycosylated hemoglobin (GHb) concentrations are a long-term indicator of blood glucose concentrations (1). Although GHb has several advantages over random fasting blood or urinary glucose measurements, GHb assessments have traditionally required blood collection techniques, which require the services of health professionals. The need for health professionals in specimen collection and preparation increases costs and reduces efficiency in care and research that involves patients in geographically scattered communities. The recent development of a mail-in filter-paper method for capillary blood samples analysis may ameliorate this problem (2–5). The advantages of this method are that venipuncture is not required, and patients can prepare and send samples from their home before data are needed. We compared this method (following the procedures provided with the kits) with our reference laboratories' microcolumn techniques.

RESEARCH DESIGN AND METHODS

Blood samples were obtained by venipuncture from 40 subjects; 38 with and 2 without insulin-dependent diabetes mellitus (IDDM). Six milliliter samples were placed in a heparinized tube, mixed, and then divided into two aliquots, each assigned a separate identification number (standards 1 and 2). At the same time, capillary blood from fingersticks was applied to two absorbent cards (2 drops/card 1 and 2), provided by Evalulab, an independent laboratory that performs only GHb testing (Palm City, FL), as part of their standard kit. Capillary blood samples were packaged according to instructions and mailed. To determine the influence of temperature variations associated with the mailing process, temperature and humidity data were collected from both the testing and analysis sites.

Laboratory A (Indiana University Diabetes Research and Training Center [DRTC]) and laboratory B (Evalulab) were blinded to the identity of the duplicate samples. Venous blood specimens were analyzed for percent glycosylated hemoglobin (HbA_{1a+b+c}) in isothermal incubation chambers by microcolumn ion-exchange chromatography via an Aldamine eliminator to remove labile glucose adducts (6) in the DRTC core immunoassay laboratory. This method is correlated with affinity chromatography in this laboratory ($r = +0.91$, $n = 16$). The interassay coefficient of variation was 2.3, 2.6, and 1.3% in 20 assays with standards measuring mean HbA_1 of 7.7, 11.3, and 16.5%, respectively. GHb was determined by the affinity chromatographic method (laboratory B) for specimens eluted from glucose oxidase–impregnated paper strips (Glyco test, Pierce, Rockford, IL). Previous reports have shown correlations of +0.97 between this method and the affinity method without filter paper. Laboratory B standardizes its values with materials provided by the University of Missouri, whose GHb values are assigned by high-performance liquid chromatography (HbA_{1c}). In a second study, to control for differences in the quantity of capillary blood placed on cards, four subjects each provided five blood samples, one for the reference laboratory and four of varying volumes for laboratory B. Laboratory B normally accepts only specimens of ≥ 20 μl, but made procedural adjustments for this test. Laboratory B reports interassay coefficients of variation 2.2, 1.0, and 1.8% for 20 assays of 3 controls having mean GHb values of 5.7, 8.4, and 12.5%.

RESULTS

Each laboratory obtained comparable results for the duplicates within its laboratory, but laboratory B's mean values were slightly lower (Table 1). The correlations between the different values obtained are shown in Ta-

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TABLE 1
Within- and between-laboratory comparisons

	Laboratory A (DRTC)		Laboratory B	
	1	2	1	2
Means ± SD (%)	10.79 ± 2.34	10.82 ± 2.34	10.23 ± 1.78	10.07 ± 1.83
Ranges	5.8–16.3	5.8–16.6	6.7–14.4	6.3–13.6

DRTC, Diabetes Research and Training Center.

ble 2. The laboratory A within-laboratory correlation was 0.98 on the duplicates, whereas the commercial within-laboratory correlation was 0.94. Both laboratories provided their own duplicate values on each of the 80 samples, and these yielded similar correlations. However, the between-laboratory correlations were lower ($r = 0.69–0.77$), suggesting that the differences between laboratories were not simply the result of a shift in mean values. These correlations were considerably lower than those obtained between the microcolumn method and affinity chromatography ($r = +0.91$).

Because two technicians supervised the application of capillary blood to the cards for the commercial assay (although all cards were saturated as instructed), the data were analyzed before and after a specific date. This yielded samples of 16 and 24 subjects. The within-laboratory correlations were not affected by this change, but the between-laboratory correlations were improved (Table 3).

Next, the data were analyzed by ranking the GHb values for each laboratory to determine whether each identified the same subjects as having poor glycemic control. Among the 10 subjects with the highest values for HbA_1 from laboratory A, 6 were also in the top 25% of the values obtained from laboratory B. The four other subjects were ranked 14th, 16th, 26th, and 27th at laboratory B.

Finally, the possibility was considered that the differences obtained were the result of varying quantities of blood provided for analysis by laboratory B. Four subjects each provided five blood samples, one venous sample for analysis by laboratory A and four samples (5, 10, 30, and 50 µl) for analysis by laboratory B. The results are shown in Table 4. Spuriously high values were always obtained from the analysis of the 5-µl samples. These values averaged 16% higher than laboratory A results and 11% higher than the mean of the 10-30-, and 50-µl samples. In no case was the result from the 5-µl sample as low as any other sample in either laboratory.

Other variables were also considered in an attempt to explain the discrepancies in the data obtained by these two methods. However, neither weather conditions (mailed samples are exposed to more extreme temperature fluctuations), outliers, time to processing, or other identifiable characteristics could account for the observed differences.

TABLE 2
Within- and between-laboratory correlations

	Laboratory A (DRTC)		Laboratory B	
	1	2	1	2
DRTC 1		0.98	0.77	0.71
DRTC 2			0.74	0.69
Commercial 1				0.94

DRTC, Diabetes Research and Training Center.

DISCUSSION

Recognizing that different methods for assessment of GHb analyze different moieties, the absence of a universally recognized GHb standard makes it difficult to draw conclusions from intermethod comparisons. However, measurement of GHb in patients with diabetes is accepted as a standard method for assessment of recent glycemic control and therefore is a critical element in clinical practice. The recent availability of a patient-prepared chromatographic method for these measurements offers attractive potential advantages in the management of these patients. In particular, the elimination of phlebotomy can enable the physician to monitor GHb more frequently without extra

TABLE 3
Within- and between-laboratory correlations by date

	Laboratory A (DRTC)		Laboratory B	
	1	2	1	2
Early samples ($n = 16$)				
Standard 1		0.98	0.84	0.82
Standard 2			0.84	0.83
Commercial 1				0.95
Late samples ($n = 24$)				
Standard 1		0.98	0.82	0.82
Standard 2			0.80	0.79
Commercial 1				0.93

DRTC, Diabetes Research and Training Center.

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TABLE 4
Effects of varying blood volume on HbA_1 results

Subject	Laboratory A (DRTC)	Laboratory B (μl)			
		5	10	30	50
1	5.72	7.01	6.44	6.60	6.46
2	15.81	17.55	15.00	15.37	15.87
3	6.27	7.51	6.63	6.62	6.55
4	11.66	12.37	12.08	11.65	11.80

Values are percentages.

office visits. The purpose of this study was to compare this newer technique with what is now the usual method for obtaining these clinical data. Although a third method (affinity chromatography) is also available, it is less frequently used in clinical practice and was used only as a comparison for the standard laboratory in this study. This new methodology has potential for use in research in the long-term monitoring of subjects whose lack of access to the research center might prevent frequent visits. The potential for bias in research is great when such patients are excluded.

The within-person variability of repeat samples from laboratory B was slightly greater than that from laboratory A. This factor has some negative connotations in that the inflation of within-person variability increases the required sample size to detect associations. Note that both laboratories are well within the National Institutes of Health criteria for intralaboratory coefficients of variation. A method might still have important clinical usefulness if it successfully ranked patients, so that a physician could classify a patient as being in good or poor glycemic control with certainty. Unfortunately, there was between-laboratory disagreement on 4 of 10 patients ranked as having the highest GHb. Thus, regardless of which laboratory was considered the standard, there would be considerable discrepancies in the identification of those in poor glycemic control. Such discrepancies in clinical care would result in much different approaches to the patients, depending on which GHb value was used.

The Evalulab system was easy to use and readily understood by patients. In addition, the assay, when prepared and processed precisely in accordance with Evalulab's instructions, was similar to but slightly more variable than the DRTC laboratory. The usefulness of this system in clinical practice would depend on the availability and costs of other laboratory services. However, for monitoring GHb between regular office visits, the use of capillary blood and mail-in sample cards might represent a convenient alternative for some patients who cannot easily provide venous blood samples. However, caution would be required if these data are compared with those collected by other methods.

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Effect of Isocaloric Substitution of Chocolate Cake for Potato in Type I Diabetic Patients

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Traditional dietary advice given to people with diabetes includes eliminating simple sugars (primarily sucrose) from the diet. Many people have difficulty following this recommendation. Because patients with type I (insulin-dependent) diabetes do not need overall calorie restriction, there is no caloric reason to restrict sucrose. In this study, we looked at the effect of the isocaloric substitution of a piece of chocolate cake for a baked

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