Temperature Control in Assay of Glycosylated Hemoglobins

To the Editor:

Recently, Dods and Bolmey (*1*) reported the use of a standard and a correction factor to overcome the consequences of uncontrolled day-to-day temperature fluctuations during ion-exchange chromatographic assays of glycosylated hemoglobins (HbA_{Ia-c}).

Table 1. Effect of Elution Temperature on HbA_{Ia-c} Value

Elution temp, °C	Mean HbA_{Ia-c}, % (SD)	
	Diabetic	Normal
18	15.63 (0.07)	6.67 (0.19)
23	16.22 (0.20)	7.59 (0.14)
28	18.49 (0.74)	8.98 (0.25)
33	22.54 (0.16)	12.11 (0.32)

Because these standards are not easily available, and because a correction factor for this analysis is highly subjective, we have constructed an assay apparatus to strictly control the operating temperature: a constant-temperature circulator forces water into a transparent Plexiglas compartment in which chromatographic columns are placed during the run.

With the use of our apparatus, the within-run CV was 1.33% for 61 samples from normal and diabetic donors, assayed in duplicate and occasionally in triplicate, at 23.0 °C (SD 0.1 °C), vs a CV of 3.03% reported by Bio-Rad Laboratories, Richmond, CA 94804, for assays without any temperature control.

Accuracy was checked by comparison with the chromatographic procedure of Saibene et al. (*2*); the correlation coefficient was 0.988 (n = 80).

We have also checked the effect of temperature on the elution profile and the result of the assay. Increasing temperature causes all hemoglobin fractions to elute faster, so that "slow" fractions partly overlap with "fast" ones, thus leading to overestimation of HbA_{Ia-c} (Table 1). Conversely, decreasing temperature leads to underestimation of HbA_{Ia-c}, owing to partial retention of "fast" fractions. The best resolution between "fast" and "slow" fractions, was obtained at 23.0 °C (SD 0.1 °C).

Apart from the dramatic effect of temperature on results with the kits supplied by Bio-Rad Laboratories, the assay is not affected, at the suggested dilution and elution volumes, by the amount of hemoglobin loaded on the column in the range of 1 to 2.7 mg, corresponding to an hemoglobin concentration in blood of 80 to 180 g/L. For greater amounts of hemoglobin, HbA_{Ia-c} is overestimated; therefore, blood samples with very high hemoglobin concentrations (>180 g/L) should be diluted before the assay.

HbA_{Ia-c} percentage, measured with our apparatus at 23.0 °C in 84 adult subjects with normal values for the oral glucose tolerance test [according to Fajans and Conn criteria (*3*)] was 6.16% (SD 0.63%), with 95% confidence limits between 4.90% and 7.42%. There were no significant differences by sex or age.

References

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