IMPROVEMENT OF GLYCOSYLATED HEMOGLOBIN MEASUREMENT
BY DISPOSABLE ION-EXCHANGE COLUMNS.*

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Glycosylated hemoglobins are a post-transcriptional modification of human hemoglobin. Their detection is a helpful tool to assess long-term glycemic control in diabetics. Several methods have been proposed for the assay of glycosylated hemoglobins, and many tests are now commercially available.

In the present work one of these tests has been evaluated, improved and compared with a reference method. Various factors affecting this glycosylated hemoglobin assay have been investigated in order to settle the best working conditions. Noticeable accuracy and precision have been obtained by monitoring the operating temperature and by taking care in the handling of the samples.

The assay of glycosylated hemoglobins (HbA1c) has been performed employing a mini-column chromatography based on the separation method of Trivelli et al. 2. This technique has been modified introducing the use of a plexiglass water thermostated compartment in order to keep a constant temperature during the elution of the columns.

Key-words: Blood storage; Diabetes mellitus; Glycosylated hemoglobins; Ion-exchange chromatography.

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Operating under controlled conditions is essential since temperature is a critical factor affecting the chromatographic separation. In the range 18-33 °C it strikingly affects both the elution profile and the calculated percentages of HbA1c. At the lower temperatures (≈ 18 °C) the fast fractions are not totally eluted, while at higher temperatures (28 to 33 °C) the elution is so fast that non-HbA1c hemoglobins are partially collected together with the fast fractions, yielding under- and overestimation, respectively. The temperature of 23 °C seemed to give best resolution and has been throughout employed.

Precision has been tested running 61 samples from normal and diabetic donors in duplicate and occasionally in triplicate. An overall coefficient of variation of 1.33 % has been obtained. Accuracy has been evaluated by comparison with a reference chromatographic method 1, running 80 samples with both methods in duplicate. The regression equation (y = 1.04x — 0.21) and the correlation coefficient (r = 0.988) showed that our method is highly accurate.

Whole blood from 3 diabetic patients and from 3 healthy volunteers has been used to investigate the effect of the storage on HbA1c levels. Our results suggest that whole blood can be stored up to 8 h at room temperature (23 °C) or up to 3 days at cold (4 °C) without altering the glycosylated hemoglobin concentrations. Otherwise, the hemolysate is stable up to 7 days at cold (4 °C) or frozen (—18 °C).

The effect of the blood total hemoglobin concentration on the HbA1c test has been studied assaying different hemolysates of the same sample. It appeared that low hemoglobin concentrations (down to 8 g/100 ml) do not alter the measurement, while high concentrations (more than 18 g/100 ml) may produce overestimation, possibly caused by insufficient capacity of the columns.

Repeatedly (3 times) washing of the RBC with isotonic saline (0.9 % NaCl) at room temperature yielded a decrease of the HbA1c percentage compared to whole blood (Δ = 5.0 %; no. = 13; p < 0.02). This decrease may be due to aspiration of cells during washing and to a non-uniform distribution of cells after centrifugation. This fact has been confirmed measuring HbA1c concentrations in small blood samples (50 µl) taken at different depth inside the centrifuge tube (data not shown).

In order to determine the normal value, glycosylated hemoglobins have been measured in 84 subjects (11-54-year-old, 62 males and 22 females) with normal response to the oGTT (50 g). A mean value of 6.16 % has been obtained (± 0.63 SD). No significant differences have been found according to sex or age.

CONCLUSIONS

Long-term monitoring of blood glucose in diabetic patients is a powerful tool in the clinical practice, and HbA1c assay is probably suitable for this purpose. The importance of this parameter requires a high degree of affidability together with routine feasibility. In this work we showed that a number of factors can interfere with the precision and accuracy of glycosylated hemoglobin detection by the presently investigated method. However, care in the storage of samples and in the control of the operating temperature may be easily obtained and lead to satisfactory results.
REFERENCES


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