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6.2.2 Quality Specifications for Glycated Hemoglobin A1c

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ABSTRACT

Berne (1) has suggested that a change in the measured value (in per cent) of GHbA1c that is smaller than 1.0% does not lead to any therapeutic action from the clinician. Since the meeting of the "Friibergh Herrgård Seminar", April 23-24, 1990 on the "Medical Need for Quality Specifications in Laboratory Medicine" (9) we have discussed this with our specialists at the Kuopio University Hospital and they share Berne's opinion. Keeping this in mind we have standardized our method so that the total analytical variation of the GHbA1c is small as compared to the measured biological variation of GHbA1c in nondiabetic and diabetic subjects. In this report we describe how we have succeeded in keeping the coefficient of variation of our routine method for GHbA1c analyses small enough for clinical purposes.

INTRODUCTION

The treatment of diabetes mellitus should be controlled so that diabetic patients may live with minimal amounts of complications for as long as possible. The determination of GHbA1c has been generally shown to fulfil the criteria of a good analyte in the follow-up of the course of diabetes. The usual assumption is that by keeping the values of GHbA1c below 8.5% in insulin treated diabetics and below 7.5% in diabetics treated with diet and/or oral antidiabetic drugs the above goal will be achieved. In this report we describe how we have succeeded in keeping the analytical variation of GHbA1c small enough for clinical purposes.

METHOD

For the measurement of glycated hemoglobin many methods have been published, but its seems quite evident that the HPLC-methods that measure only the proportion of glycated hemoglobin A1c or affinity chromatography which measures all the glycated forms of hemoglobin (GHb), are the most useful in routine clinical laboratory work (2,6,7,9). In the university central hospital district of Kuopio we have used the FPLC-system of Pharmacia (Uppsala, Sweden) for the measurement of GHbA1c in blood. To control the analytical level of our method we have used washed and concentrated frozen (-70°C) erythrocytes (1) to which potassium cyanide was added to improve the stability of the samples. The method measures glycated HbA1c and acetylated HbA1c (10) and is reasonable linear between 3.0 and 20 %. When carefully standardized for the assay conditions it is not influenced by HbF thus also suitable for analyses during pregnancy (5). To improve the quality of the HPLC methods it is essential that the peak integration is performed properly. According to Jeppson (5) the valley-to-valley method should be selected for quantitation. We have modified our runs so that the baseline of the runs is quite stable. This allows the calculation of the results on the basis of the base line method (Fig. 1).

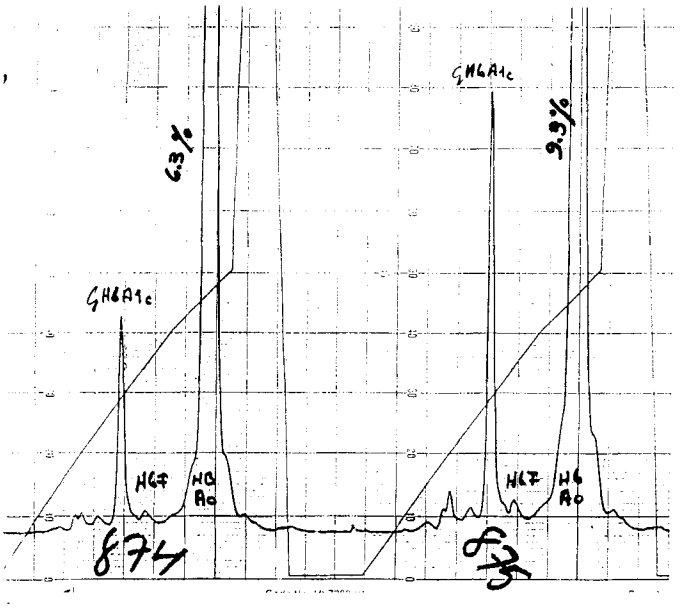

Figure 1. Typical runs of GHbA1c from samples from a healthy and a diabetic patient by our FPLC-method (Pharmacia, Uppsala, Sweden).

The accuracy of the present method was studied by measuring GHbA1c values during three working weeks from a frozen sample stored at -70°C. The mean and the coefficient of variation for the 15 measurement points was 5.86 % and 1.15 %, respectively. We have studied our method by standardizing all phases of it to diminish the variation. The coefficients of variation for within day and between days are presented in Table 1.

Table 1. The results of GHbA1c measurements within series and between series from a pooled sample stored at 70°C analyzed by Mono S column (Pharmacia, Sweden). The biological variation of GHbA1c from 11 healthy individuals at normal level is also shown.

Variation type	No. of analyses	GHbA1c %	S.D. %	CV %
Within series	20	7.69	0.076	0.98
Between series	20	7.59	0.107	1.41
Biological	11	5.38	0.34	6.35

The number of these measurements was only 20 and it is assumed that the coefficients of variation would be smaller when larger number of analyses had been measured. The biological variation was studied for 11 non diabetic subjects to avoid the possible effects of the treatment to the values (ten analyses from each). The value 0.34 % as the standard deviation of the biological variation for the mean of 5.36% was also quite acceptable and corresponded well with our reference interval for healthy individuals 4.0-6.0 % (0.34 × 3 × 2 ≈2.0 %).

RESULTS AND DISCUSSION

Assuming that there was no bias in the method, from the coefficients of variation of within (s_{Aw}) and between (s_{Ab}) series presented in Table 1 the total analytic variation of the method (s_A) can be calculated as follows (3): $s_A^2 = s_{Aw}^2 + s_{Ab}^2$. The calculated total analytical variation (s_A) at the critical GHbA1c level of 7.6% was 1.72 % from the values of s_{Aw} = 0.98% and s_{Ab} = 1.41% The biological variation of 11 nondiabetic subjects as standard deviation at the normal level was 0.34%, which is smaller than that presented earlier (4). From these values the total variation (s_T) including the analytical (s_A) and the biological variation (s_B) can be calculated and was 0.36% according to the formula s_T^2 =

$s_B^2 + s_A^2$. This indicates that the main source of error in the GHbA1c values is due to the biological variation, being about 1/3 of the 1.0% variation tolerated by our clinicians before reacting to changes in GHbA1c level. Thus the method used is working very suitable for clinical purposes.

The reduction of the analytical variation from the present values will not affect very much the total variation because the biological variation is so marked. On the other hand, the methodological bias, when present, can considerably increase the total variation of the GHbA1c method. It is hoped that in the future a stable and pure calibrator with known values for both GHbA1c and GHb will be available (8). This should allow the comparison of the different methods being currently used.

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