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# Quality Control and Quality Requirements for the Measurement of Glycated Hemoglobin

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In the follow-up of the treatment of diabetes mellitus the determination of glycated blood proteins, especially blood hemoglobin, has been proved to be very important in estimation of the long-term balance of the disease. Many methods have been developed, but its seems quite evident that the HPLC-methods which measure the proportion of glycated hemoglobin A1c (GHbA1c) or the affinity chromatography measuring all glycated forms of hemoglobin (GHb) are the most useful for the routine clinical laboratory work (1,2,3,5).

There are many difficulties in organizing the quality control of the determination of glycated hemoglobin. First problem e.g. in Finland is that there are at least six commercial methods in use. In Table 1 there are presented the methods and their reference intervals for the determination of glycated hemoglobins. The second is the measurement temperature, which must be know when the values for glycated hemoglobins has been calculated by using minicolumn techniques. The best way is to perform the columnphase in a thermostated water bath. The third and perhaps the most difficult problem is to find the ideal control material which fulfills the criteria: the control material should be identical with patient samples, the concentration of the analyte should be at the desired level and the stability of the control material should be long enough.

In the university central hospital district of Kuopio we have selected an automated HPLC-method and measured GHbA1c values for the whole district. Today we have two different instruments for the measurement of GHbA1c: the older one (3) is the FPLC-system (Pharmacia, Uppsala, Sweden) and the newer one an HPLC-instrument of Shimadzu (6) with an strong ion-exchange

column supplied by Beckman Instruments Inc. (Palo Alto, CA, U.S.A.).To follow-up the analytical level of our method we have used washed and frozen (-70°C) concentrated erythrocytes (1). The typical variation coefficients within day and also between days have been between 1.5 and 2.0 %. Our method measures glycated HbA1c and acetyl HbA0 (3,7), it is well linear between 3.0 and 20 % and is not influenced by HbF thus being suitable for analyses during pregnancy.

In the meetings with our clinicians it has been stated that the difference of the GHbA1c value measured should not exceed 0.5 % from the right value in the normal reference interval of 4.0-6.0 %. This means that the analytical variation should be below 2.5 %. It seems that this can be reached when the method is carefully selected, calibrated and continuously followed. We believe that this these criteria will be obtained in most laboratories where automated HPLC-techniques for GHbA1c is used.From the clinical point of view it is almost important that on a district, where the diabetics visit to different physicians and thus to many laboratories, an agreement on the principle (one method) is made for clinical use.

The more difficult question concerns the right value when performing the quality control surveys in Finland and in Nordic countries. The controls should contain both the stabile ketoamine form as well as the labile Schiff's base so that they correspond as closely as possible the situation in clinical laboratories. The first step in the measurement of GHbA1 or GHbA1c is the elimination the labile Schiff's base, which it is not so marked when using affinity chromatography for GHb (4). For this reason lyophilized hemolysates may give too good results when using minicolumns and ion-exchange technique. Secondly many commercial lyophilized preparations contain components or fractions which render the HPLC-techniques (1). In most surveys we have seen (Table 2, Fig. 1) that the HPLC-methods as a whole are not very much better in performance than the manual methods using either affinity or ionexchange minicolumn. It can, however, be calculated that EDTA-blood samples are better than the hemolysates (Fig. 1) and the smallest coefficient of variation is seen in the group of HPLC-methods especially when the variation coefficients are

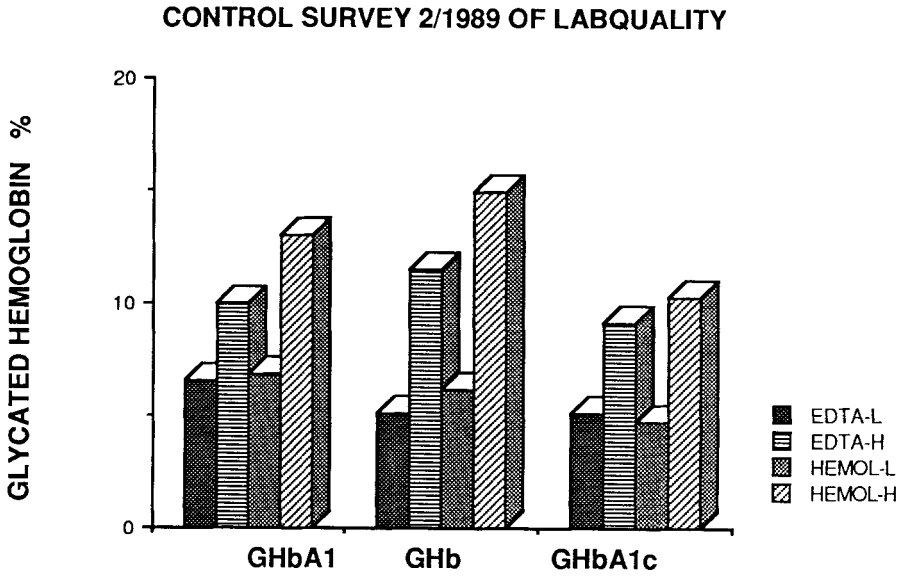
Table 1. Different methods used by participating laboratories in the the surveys of Labquality Ltd. for the determination of glycated hemoglobins and they reference intervals

| Method | Component measured | Reference intervals |
|---|---|---|
| Isoelectric focusing | GHbA1c | 4.0 - 6.5 % |
| HPLC/FPLC ionex chromat. | Acetyl HbA1c | 4.0 - 6.1 % |
| Mini-ionex chromat./ Biorad | (HbF) | 3.8 - 6.1 % |
| Electrophoresis | GHbA1a,b,c | 5.5 - 9.0 % |
| Mini ionex chromat. | Acetyl HbA1c<br>HbF<br>(Other Hb:s) | 5.6 - 8.6 % |
| Mini affinity chromatography | GHbA1a,b,c<br>GHbF<br>GHbA2<br>GHBAO | 4.6 - 8.2 % |

Table 2. The quality control results from the surveys during the years 1987 to 1989 by Labquality Ltd. in Finland. The number of laboratories using mini-column techniques (GHbA1) has decreased, the users of affinity chromatography (GHb) correspondingly increased and those with HPLC-techniques (GHbA1c) clearly increased.

| Method | Sample | No. | Mean | SD | CV |
|---|---|---|---|---|---|
| GHbA1 | EDTA-blood/1/87 | 21 | 6.1 % | 0.45 | 7.3 % |
| | EDTA-blood/1/88 | 20 | 10.5 % | 0.77 | 7.3 % |
| | EDTA-blood/1/89 | 14 | 6.5 % | 0.89 | 13.7 % |
| | Hemolysate/1/87 | 21 | 7.5 % | 0.89 | 11.9 % |
| | Hemolysate/1/88 | 19 | 12.8 % | 0.49 | 10.0 % |
| | Hemolysate/1/89 | 14 | 5.3 % | 0.74 | 10.7 % |
| GHb | EDTA-blood/1/87 | 8 | 4.9 % | 0.51 | 10.4 % |
| | EDTA-blood/1/88 | 8 | 13.7 % | 1.07 | 7.8 % |
| | EDTA-blood/1/89 | 13 | 9.95 % | 0.52 | 10.0 % |
| | Hemolysate/1/87 | 8 | 5.0 % | 0.64 | 12.8 % |
| | Hemolysate/1/88 | 8 | 13.6 % | 1.57 | 11.6 % |
| | Hemolysate/1/89 | 13 | 5.3 % | 0.94 | 17.9 % |
| GHbA1c | EDTA-blood/1/87 | 5 | 4.7 % | 0.49 | 10.5 % |
| | EDTA-blood/1/88 | 6 | 9.8 % | 0.35 | 3.6 % |
| | EDTA-blood/1/89 | 22 | 4.8 % | 1.04 | 14.7 % |
| | Hemolysate/1/87 | 5 | 5.1 % | 0.11 | 2.2 % |
| | Hemolysate/1/88 | 6 | 10.5 % | 0.45 | 4.5 % |
| | Hemolysate/1/89 | 22 | 5.1 % | 0.77 | 14.9 % |

Fig. 1. The results from the quality control surveys of glycated hemoglobin by three most common methods in Finland. The results are expressed as the mean values in the survey 2/1989.



calculated separately for each Nordic country.

As stated before it seems possible to maintain the intralaboratory variation good enough, eg. below 3.0 %. However, when the interlaboratory variation is much more higher, it seems that there must be troubles either in quality control samples or in the standardization. The great importance of the value of glycated hemoglobin in the treatment of diabetics and on the other hand possible technical difficulties in the measuring procedures, according to our opinion, is a good reason to start a Nordic project.

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