Comparison of five commercial kits for the determination of glycosylated haemoglobin

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SUMMARY Five commercial chromatography kits for the measurement of glycosylated haemoglobin have been compared using blood specimens obtained from diabetic patients and from non-diabetic controls. Assessment was based on accuracy, general impressions of practicality, and overall performance. The precision of each technique was assessed by selecting five diabetic and five non-diabetic specimens and testing each one by each technique. All systems tested were of a high standard and the advantages and disadvantages of each system are discussed.

Glycosylated haemoglobin (HbA₁) is a minor component of normal adult haemoglobin (HbA), and in healthy subjects constitutes about 7% of the total haemoglobin. It consists of several sub-fractions¹⁻³: HbA_{1a}, HbA_{1b}, and HbA_{1c}. HbA_{1c} is the major sub-fraction and accounts for roughly 80% of glycosylated haemoglobin. All three sub-fractions are increased proportionately in patients with diabetes mellitus,⁴ and the total amount of HbA₁ is thought to be a good indicator of long term metabolic control.

Most commercial kit methods for measurement of HbA₁ are chromatographic and, compared with other methods, these have the disadvantage of being temperature dependent. Furthermore, not all commercial kits give the user the option of eliminating aldimine, which is the unstable intermediate of the glycosylation reaction. Since the aldimine fraction normally accounts for less than 10% of HbA_{1c} it has been suggested that the elimination is generally unnecessary unless an acute change in blood glucose concentration has recently occurred⁵ or the patient is uraemic or has a history of alcoholism.

Material and methods

Five commercial chromatography kits for the measurement of glycosylated haemoglobin were assessed and compared. The kits used were HbA₁ Test Combination (BCL, Boehringer Corporation), Bio Rad (Bio Rad Laboratories Ltd), DCA Stat Glycos (IRE Ltd), Isolab Quick-Sep (Advanced Laboratory Techniques), and Sigma (Sigma Corporation). All

were 100 test size, though some of the manufacturers produced 20 test sizes. Assessment was based on several factors: accuracy and precision, general impressions of practicality, and overall performance.

Blood specimens were obtained from diabetic patients at various levels of control and from known non-diabetic subjects. HbA₁ concentration was determined within the stipulated time period of each kit—that is, all samples tested were less than one week old and had been stored at 4°C. Edetic acid anticoagulated blood was used throughout. Each batch of tests was run simultaneously under the same temperature conditions and using the same specimens.

Non-diabetic subjects were chosen from laboratory staff and hospital inpatients with various disorders; care was taken so as not to select those who were known diabetics. All results are summarised in Tables 1-4.

A precision check of each technique was carried out by selecting specimens from five diabetic and five non-diabetic patients. Each specimen was tested by each technique. Thus each technique was subjected to a precision check by allowing each sample to be assayed five times simultaneously under identical conditions. The results are shown in Table 2.

Table 1 *Results of tests on controls*

Kit	No of samples	Mean HbA ₁ %	Standard deviation	Range Mean $\pm$ 2SD
BCL	51	5.3	1.08	3.1-7.5
Bio Rad	51	5.85	1.40	3.0-8.7
DCA	51	5.9	1.18	3.6-8.8
Isolab	51	5.3	1.10	3.1-7.5
Sigma	51	5.7	1.62	2.4-8.9

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Table 2 Results of precision check

Kit	No of samples		Mean HbA ₁ %		Standard deviation		Coefficient of variation	
	ND	D	ND	D	ND	D	ND	D
BCL	10	10	5.1	13.1	0.10	0.24	1.94	1.80
Bio Rad	10	10	5.1	10.3	0.39	0.29	7.60	2.80
DCA	10	10	4.8	12.8	0.33	1.00	6.80	7.85
Isolab	10	10	3.7	10.6	0.24	0.43	6.43	4.00
Sigma	10	10	4.8	18.2	0.46	1.31	9.50	7.18

ND = non-diabetic, D = diabetic.

METHODS

BCL HbA₁ Test Combination

The BCL (HbA₁ Test Combination) kit consists of 100 micro disposable columns prefilled with resin, a bottle each of lysing agent, first elution buffer, second elution buffer, and an implement for releasing the seal on each column.

Fifty microlitres of whole blood is added to 200 μ l of lysing agent, mixed, and allowed to stand for 10 min. During this time the temperature of the environment is noted.

The excess buffer is drained through the column, which contains a filter disc to stop it drying out, and 50 μ l of haemolysate is added to the top of the column bed. Penetration of the lysate into the column rapidly occurs, and 200 μ l of the first elution buffer is then added. Once this has penetrated the column a collection tube is placed underneath and 4 ml of the first elution buffer is added in order to elute the glycosylated fraction. Collection of this fraction is complete in about 15 min, after which the collection tube is changed and 4 ml of the second elution buffer is added to the column, which elutes the remainder of the haemolysate.

Once complete the second fraction is diluted with distilled water and the absorbance of each fraction is measured in a spectrophotometer at a wavelength of 415 nm. The glycosylated fraction is then calculated as a percentage of the total after making a correction for temperature using the chart provided. If a BCL rack is used in conjunction with a water bath at 23°C, however, conversion to the appropriate temperature is not required.

Bio Rad kit

The Bio Rad kit contains 100 prefilled disposable columns and two one litre bottles of elution/developing reagent, together with a bottle of lysing agent.

One hundred microlitres of whole blood is added to 500 μ l of lysing agent, mixed, and allowed to stand. The columns are prepared by shaking, in order to resuspend the resin, and releasing the seals

on the top and bottom. The excess buffer is then allowed to drain from the columns.

One hundred microlitres of the lysate is added directly on to the surface of the resin bed and allowed to penetrate. A collection tube is placed underneath and 10 ml of elution/developing reagent is added. While the glycosylated fraction is eluting a "total" dilution of the lysate is made by adding 100 μ l to 10 ml of elution/developing reagent.

The elution process is complete in about 45 min and the absorbance of both fractions is read in a spectrophotometer at a wavelength of 415 nm. The percentage of glycosylated haemoglobin is thus calculated and no correction is made for temperature. A temperature controlled rack is available from Bio Rad.

DCA Stat Glycos kit

The DCA Stat Glycos kit is a unique system consisting of a single bottle of resin and 100 tubes and separators, which are reusable. A bottle of lysing agent sufficient for 100 tests and a lyophilised specimen of a standard and control are also included. Once reconstituted the standard and control will store in aliquots of 100 μ l for two months at -20°C. The value of the standard and the range of the control are printed on each vial.

One hundred microlitres of whole blood is added to 500 μ l of lysing agent and allowed to stand. Three millilitres of well mixed resin is pipetted into the tubes provided and 100 μ l of the lysate is added. The filter separator is then placed in each tube and mixed continually for 10 min.

After mixing, the glycosylated fraction—that is, the fraction not bound to the resin—is eluted by pushing the filter separator towards the bottom of the tube. The eluate may then be poured directly from the separator into the spectrophotometer for the absorbance reading at 415 nm wavelength. The absorbance of the "total" fraction made by adding 20 μ l of lysate to 5 ml of distilled water is also measured and the percentage of glycosylated haemoglobin is calculated.

The calculated value of the standard and control is compared with the stated values and the correction factor obtained. This correction factor is then applied to the test results, thus eliminating the need for temperature correction.

Isolab kit

The Isolab kit consists of 100 prefilled disposable columns, first and second elution reagents, and a bottle of lysing agent. Also included is a bottle of reagent which, when added to the lysate, will eliminate the aldimine fraction. Hence the user is provided with a choice of whether to eliminate the aldimine or not. In this trial the eliminator was not used.

Fifty microlitres of whole blood is added to 200 μ l of lysing agent and allowed to stand. The excess buffer is drained through the column and the top of the filter disc, which prevents drying of the column, is positioned on the surface of the bed. Fifty microlitres of lysate is added to the column and once penetration has occurred a further 200 μ l of the first elution buffer is added and allowed to drain. A collection tube is then placed underneath and 4 ml of the first elution buffer is added to the column. The eluate collected is thus the glycosylated fraction and this step is complete in about 20 min. The collection tube is then changed and 4 ml of the second solution is added. This releases the remaining haemoglobins from the column, which are then diluted with distilled water. The absorbance of both fractions is read on a spectrophotometer at a wavelength of 415 nm and the percentage of glycosylated haemoglobin is calculated. This is then amended by adjusting the results to correspond with the environmental temperature.

Table 3 Results of tests on diabetic patients

Kit	No of samples	Mean HbA _{1c} %	Standard deviation	Range Mean $\pm$ 2SD
BCL	90	10.05	2.16	5.8–14.1
Bio Rad	90	9.4	1.97	5.4–13.3
DCA	90	7.5	1.76	4.0–11.0
Isolab	90	9.5	2.04	5.4–13.6
Sigma	90	9.3	2.26	4.8–13.8

Table 4 Differentiation between non-diabetic and diabetic values

Kit	% Overlap of HbA _{1c} in diabetic and non-diabetic values
BCL	15.0
Bio Rad	32.0
DCA	64.8
Isolab	20.0
Sigma	35.9

Sigma kit

The Sigma kit consists of 100 prefilled disposable columns, a single bottle of buffer, and a bottle of lysing agent. The columns may, if required, be prepared by shaking to resuspend the resin or by simply draining off the excess buffer through the column, though the latter method may prolong the technique by a few minutes.

One hundred microlitres of whole blood is added to 500 μ l of lysing agent and allowed to stand after mixing. Fifty microlitres of this lysate is then added directly on to the resin bed surface and allowed to enter. A collection tube is then placed underneath the column and 5 ml of the buffer solution carefully added so as not to disturb the resin, and the eluate is collected. The process is complete in about 15 min, thus allowing time for a dilution of the total haemoglobin to be made by adding 20 μ l of lysate to 10 ml of the buffer.

The absorbance of both fractions is read in a spectrophotometer at a wavelength of 415 nm and the percentage of glycosylated haemoglobin is calculated. A correction factor is used in calculating the final result in order to compensate for environmental temperature.

General impressions

The BCL kit is easy to use and the inclusion of a simple implement for releasing the seals on the columns is particularly useful. A filter disc prevents the column from drying out and keeps the resin compact when adding the reagents, one of the similarities to the Isolab technique. The temperature correction chart, however, accounts for temperatures no less than 20°C, which during certain times of the year may be difficult to maintain. This may be overcome by use of the BCL temperature controlled rack.

The Bio Rad kit has the distinction of being the first commercial assay kit for glycosylated haemoglobin on the UK market. It contains a comprehensive instruction booklet, which includes a short history of the present technique, together with many references and the normal ranges produced by many workers. Unfortunately, however, it does not contain facilities for correction due to temperature differences, but recommends that the test be carried out at 22°C. Despite this, the kit is easy to use and a reliable result may be obtained within 1 h. From the point of view of comparison it is unfortunate that the laboratory was not aware of the existence of the Bio Rad temperature controlling rack. It should be noted that the trial involved the Bio Rad HbA_{1c} kit and not the Bio Rad A_{1c} specific kit, which is now marketed.

The DCA kit is the cheapest of those tested and

Normal and diabetic ranges of each technique. Shaded areas represent overlap between the two ranges.

the cost may be reduced even further after the initial purchase of the first kit as the tubes and filter separators are reusable. The kit is easy to use and is the only technique of those studied which does not involve a sub-column collection. A result may be obtained within 30 min, and since the kit contains standard and control no correction for temperature per se is required, but an overall correction factor is applied.

The Isolab system is remarkably similar to that of BCL in appearance. It contains a filter disc on the surface of the resin bed to prevent drying out and to aid the addition of buffers. The temperature correction chart provided covers a range from 18–28°C and hence is more adaptable to a particular environment than BCL. It also contains an aldimine eliminator to be added, if required, at the users discretion.

The Sigma technique is quick, producing a result within 45 min. The kit is easy to use and the temper-

ature correction factor makes the calculation of results relatively easy and eliminates the use of a correction chart.

Discussion

All systems tested were of a high standard and difficulty in choosing any particular one inevitably occurred. The choice will vary between establishments depending on the anticipated number and the budget available.

The BCL system showed excellent precision with coefficients of variation of 1.8% on a specimen with a HbA₁ concentration in the diabetic range and 1.9% in the normal range. The Figure shows BCL to have the smallest overlap area of those tested. It was 15.0% of the total ranges, and hence shows more differentiation between the two ranges than any of the other techniques.

The Bio Rad system compares remarkably well despite the fact that temperature correction facilities were not used. An overlap of ranges of 32% is shown in the Figure.

The DCA system is inexpensive and easy to use but unfortunately performed rather disappointingly on the precision check. It also had an overlap of ranges of 64.8% (Figure). It is the only system of those tested which includes standards and controls.

The Isolab system shows mid-range precision performance. The inclusion of the filter disc and the wide temperature range in which it can be used make it versatile. The Figure shows a 20% overlap of ranges, which compares well with its competitors.

The Sigma system performed disappointingly, with a coefficient of variation 9.5% in the normal range and an overlap of ranges of 35.9%. Despite the ease of use and speed, it would probably not be chosen as the ideal system.

The sum of the values obtained for coefficient of variation in both ranges and the percentage overlap of ranges is expressed as the "summary index" and is shown in Table 5.

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Table 5 Summary of results

	Coefficient of variation (%)		% Overlap of ranges	"Summary index"
	Non-diabetic	Diabetic		
BCL	1.94	1.80	15.0	18.74
Isolab	6.43	4.00	20.0	30.43
Bio Rad	7.60	2.80	32.0	42.40
Sigma	9.50	7.18	35.9	52.58
DCA	6.80	7.85	64.8	79.45

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