A report on the interlaboratory quantitation of haemoglobin A₂ and haemoglobin F

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SYNOPSIS An interlaboratory trial of the quantitation of Hb A₂ and Hb F has been carried out on two samples of blood by 90 laboratories in the British Isles. The results have been compared with those obtained by four reference laboratories. Overall, the correlation was very poor and indicated that the methods used have to be standardized before interlaboratory values become meaningful. Moreover, in many laboratories the level of Hb A₂ in a patient with proven beta thalassaemia was not reported as being increased.

Haemoglobin A₂ (Hb A₂) and haemoglobin F (Hb F) are minor components of the haemoglobin of human red cells. In normal subjects the proportion of each does not show much variation: Hb A₂ 1.5-3.2% (Weatherall, Gilles, Clegg, Blankson, Mustafa, Boi-Doku, and Chaudhury, 1971), Hb F 0.8 ± 0.03% (Weatherall and Clegg, 1972; Lehmann and Huntsman, 1972).

At the 14th Congress of the International Society of Hematology in Sao Paulo in 1972, the International Committee for Standardization in Hematology established an expert panel on the subject of 'Screening procedures for the recognition of abnormal haemoglobins. As the presence of elevated Hb A₂ and Hb F is significant in making a diagnosis of heterozygous alpha or beta thalassaemia, it is essential to be able to measure these haemoglobins reliably. This will be an important aspect of the work of the ICSH panel.

Although in many laboratories normal ranges are established with the technique used it is not known to what degree individual values vary between laboratories. Neither is it known whether all laboratories would be able to detect minor alterations. To answer these questions it was decided to carry out a pilot survey using the organization of the National Haematology Proficiency Testing Project of the British Committee for Standards in Haematology (BCSH) and the Department of Health and Social Security (DHSS).

Protocol

A REFEREE LABORATORIES

Four laboratories which are actively concerned with

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investigation of abnormal haemoglobins and regularly quantitate Hb A₂ and Hb F served as referee laboratories. Before the trial normal and abnormal blood samples were sent to each. The results shown indicated that agreement was close enough for all four laboratories to act as referee centres for the trial.

B TEST LABORATORIES

One hundred and sixty laboratories throughout the United Kingdom agreed to participate. These are referred to as the test laboratories.

C BLOOD

One unit of fresh blood (A) from a normal donor and one unit of blood (B) from a volunteer patient with beta-thalassaemia trait in whom the diagnosis had been proven by measuring the relative rates of chain synthesis were used. The blood was bottled in 3 ml aliquots in EDTA and distributed to the various laboratories, including the four referee laboratories. Participants were asked to measure the Hb A₂ and Hb F and to indicate the date and the method used. Ninety laboratories reported their results.

Results

REFEREE LABORATORIES

These are shown in the table. The mean values for sample A were: Hb A₂ 2.3 ± 0.6%, Hb F 0.83 ± 0.03%. The mean values for sample B were, Hb A₂ 4.6 ± 0.3%, Hb F 0.88 ± 0.36%. The ratio analysis of these results was 0.52 ± 0.05 indicating that the variability of these results was probably due to a reproducible error in the technique.

Laboratory No.	Sample A		Sample B	
	A ₂ (%)	F(%)	A ₂ (%)	F(%)
1	2.3	0.7	3.8	0.7
	2.2	0.8	4.4	0.8
	2.4	0.8	4.2	0.8
	2.4	0.7	4.8	0.8
	2.3	—	4.3	0.8
2	2.8	0.5	5.0	0.8
	2.7	0.5	4.8	0.6
			5.0	
			5.0	
3	2.5	1.0	4.4	0.8
	2.3	0.8	4.6	0.7
	2.3	0.8	4.3	0.8
	2.0	1.0	4.2	1.0
	—	8.0	4.1	0.9
4			4.6	1.0
	2.7	1.0	5.0	1.1
	2.5	1.1	5.0	1.0
	2.5	0.8	4.9	1.0
	2.7	0.6	4.7	1.0
	—	0.9		

Table Values of Hb A₂ and Hb F obtained by the referee laboratories

TEST LABORATORIES

The distribution of reported values of Hb A₂ of samples A and B are shown in fig 1, and of Hb F shown in figure 2. For Hb A₂ the values obtained ranged from 0 to 7.5% for sample A and 0.1 to 15% for sample B. Fifty per cent of the results were within 2 SD of the true values for sample A, but only 32% for sample B.

For Hb F, 46% of the results were within 2 SD for sample A and 42% for sample B. However, many

laboratories reported values of less than 0.1% and in some a value of 0% was reported.

Figures 3 and 4 show the ratio analysis of the results of the two specimens for Hb A₂ and Hb F respectively. For Hb A₂ only the results from 15 of the laboratories were within the permitted 2 SD, the rest being widely scattered. This indicates that for any given laboratory the error in the technique was not reproducible but completely random. The same analysis of the Hb F results shows less scatter but there is a large grouping below the 1% level and it is not certain how meaningful the results are.

There is no correlation between the method used and the results. Ninety-five per cent of the test laboratories used the cellulose acetate method for the quantitation of Hb A₂ (Marengo-Rowe, 1965), some with slight modification, and for Hb F 80% used Singer's technique (Singer, Chernoff, and Singer, 1951) or the modification used by Betke, Marti, and Schilt (1959) or Pembrey and Weatherall (cited in Weatherall and Clegg, 1972). There was also no correlation between the values reported and the date at which the tests were carried out.

Conclusions

It appears from this pilot survey that there is a major problem in the interlaboratory quantitation of these minor haemoglobins. Moreover, while each laboratory may have its own normal range the ratio analysis of the results would indicate that such ranges might not be meaningful. The techniques for quantitation of these haemoglobins are not simple and even the

Fig 1 The values of Hb A₂ on samples A and B reported by the test laboratories ● compared with the values reported by the referee laboratories ○.

Fig 2 The values of Hb F on samples A and B reported by the test laboratories ●, compared with the values reported by the referee laboratories ○.

Fig 3

Fig 4

Fig 3 A comparison between the values of Hb A_2 on samples A and B reported by the test laboratories and the ideal line ± 2 SD obtained from the referee laboratories. If the reported values of any particular laboratory are higher or lower than the true values due to a constant error, the ratio of B/A should lie within the limits of the line.

Fig 4 A comparison between the values of Hb F on samples A and B reported by the test laboratories and the ideal line ± 2 SD obtained from the reference laboratories. For interpretation see legend to figure 3.

referee laboratories admit that on occasion they run into problems. As can be seen in table I the results reported from the referee laboratories are far from perfect. In our own hospital it is found that a technician needs at least a week's training on normal samples before the results become consistent. This is supported by the results obtained from test laboratories; generally the bigger the hospital the better the results, but this was not always the case.

There are a number of reasons for discrepant results. Electrophoretic separation or cellulose acetate membranes may be affected by the quality of the membrane and by the method of preparing the lysate. Unwashed cells give falsely high values. Methods in which the separated components are stained and then measured by densitometry are subject to gross inaccuracies in the presence of a non-haem protein which moves with Hb A₂.

The finding that 50% of the laboratories found normal Hb A₂ values on sample B is of some concern. The patient has the beta thalassaemia trait and is the father of two children with beta thalassaemia major. His Hb is 11.5-12.0 g/100 ml with an MCH of 23 pg. It has been proposed that in the interests of public health at a national and international level screening programmes for beta thalassaemia should be undertaken. As such programmes depend largely on the levels of Hb A₂

and F, it would seem from this pilot survey that they would be of little value without better proficiency control. There is also a need for the adoption of a standard method to ensure consistent results.

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