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A survey of current United Kingdom practice for antenatal screening for inherited disorders of globin chain synthesis

Barbara J Bain, Claire Chapman, on behalf of the UK Forum for Haemoglobin Disorders

Abstract

***Aims*—To document current United Kingdom practice for antenatal screening for inherited disorders of globin chain synthesis and to compare such practice with guidelines published by the British Committee for Standards in Haematology and the Standing Committee on Sickle Cell, Thalassaemia and other Haemoglobinopathies (SMAC).**

***Methods*—The members of the UK Forum on Haemoglobin Disorders were surveyed about their current practice for antenatal haemoglobinopathy screening. The UK Forum is a national group of haematologists, paediatricians, laboratory scientists, and counsellors working in the field of diagnosis and management of disorders of haemoglobin synthesis; such disorders including the α and β thalassaemias, sickle cell disease, and other haemoglobinopathies.**

***Results*—Completed questionnaires from 38 hospitals (or cooperating groups of hospitals) were analysed. The great majority of hospitals were applying appropriate laboratory methods, but problems were commonly encountered in ensuring that appropriate testing of antenatal patients and, when necessary, of their partners, was carried out early in pregnancy. When screening was selective there was quite often a failure to identify all women in whom testing was indicated, and cut off points used as an indication for further testing were sometimes inappropriate.**

***Conclusions*—Many practical problems are still encountered in following guidelines for the antenatal diagnosis of haemoglobinopathies. A need for improved administrative procedures and increased funding was identified. In addition there is a need for agreed guidelines giving more specific advice on technical aspects of laboratory practice.**

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Keywords: haemoglobinopathy; thalassaemia; sickle cell disease; antenatal screening

UK Forum on Haemoglobin Disorders, Department of Haematology, The Leicester Royal Infirmary Children's Hospital, Leicester, UK (Honorary Secretary, Dr C Haworth)
B J Bain
C Chapman

Correspondence to:
Dr B J Bain, Department of Haematology, St Mary's Hospital, Praed Street, London W2 1NY, UK.

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The inherited disorders of globin chain synthesis are responsible for considerable morbidity and mortality worldwide. β Thalassaemia major is incompatible with life unless the patient is regularly transfused. Transfusion in turn necessitates iron chelation treatment to avoid the serious complications of iron overload. Chelation treatment is uncomfortable, inconvenient, and expensive. Most potential parents, if fully informed, choose to terminate a pregnancy when it is predicted that the fetus will suffer from this disorder. Among the α thalassaemia syndromes, haemoglobin H disease causes a chronic haemolytic anaemia but life expectancy is little altered and quality of life is good. This condition is therefore not usually considered an indication for termination of pregnancy. The most severe α thalassaemia syndrome, haemoglobin Barts hydrops fetalis, not only leads to a non-viable fetus with death either in utero or in the first few hours after birth but is also associated with complications of pregnancy such as hydramnios and hypertension. Termination of pregnancy is therefore almost universally requested when it is predicted that the fetus will suffer from this condition. Sickle cell disease (sickle cell anaemia and compound heterozygous states associated with sickling) is a chronic painful condition which is associated with significant morbidity and considerably reduced life expectancy even in countries where optimal or near optimal medical care can be offered. A significant proportion of potential parents choose to terminate a pregnancy when sickle cell disease is predicted, particularly the most severe forms such as sickle cell anaemia (homozygosity for haemoglobin S) and sickle cell/β thalassaemia. Even those who do not wish to consider termination of pregnancy often wish to be fully informed in advance of the likelihood of a serious haemoglobinopathy.

The prevalence of thalassaemias and of variant haemoglobins varies considerably between different parts of Britain. In recent years antenatal testing for prediction of serious haemoglobinopathies has been increasingly practised in the United Kingdom and, since the publication of the report of the Standing Committee on Sickle Cell, Thalassaemia and other Haemoglobinopathies (SMAC)[1] universal screening for these disorders has increasingly been introduced in high prevalence areas. It therefore seemed opportune to review current practice and to establish whether appropriate policies were being followed. The SMAC report recommends universal screening for thalassaemia on the basis of the mean cell haemoglobin (MCH). For sickle cell disease, selective screening is recommended when the antenatal population includes less than 15% of ethnic minorities at risk of sickle cell disease, and universal screening if the antenatal population includes more than 15% at risk. The British Committee for Standards in Haematology

Table 1 The number of antenatal patients tested yearly in the centres surveyed

Number of patients	*Number of centres*
2000	1
3000	6
4000	13
5000	11
6000	3
7000	1

(BCSH) has also published guidelines for the diagnosis of disorders of globin chain synthesis.[2]

The UK Forum for Haemoglobin Disorders is a national organisation bringing together medical, paramedical, and technical staff responsible for delivering a service to those with inherited disorders of globin genes. This group decided to carry out such a survey. Centres surveyed included major hospitals in all the areas of the United Kingdom where there are significant numbers of individuals at risk of these disorders (appendix 1). Those surveyed were self selected in that they were sufficiently interested and committed to be active members of the UK Forum. This survey may therefore be seen as documenting current best practice, and any deficiencies uncovered are likely to be at least equally prevalent in centres not included in the survey.

Methods

We prepared a questionnaire which was modified and approved by a meeting of the regional representatives of the UK Forum before being circulated to members. Seventy questionnaires were distributed and 42 were returned. Since there are often two or three members of the UK Forum working in the same hospital or laboratory, the replies represented the great majority of members. Two forms were returned from hospitals with no obstetric service, and two were notifications that duplicate returns were being avoided, leaving 38 completed questionnaires for analysis. The results of the survey were analysed by us and were presented at a national meeting of the UK Forum in October 1996. Following discussion at this meeting, nine participants were contacted to provide further details or to clarify answers before the final analysis was carried out.

Results

Completed questionnaires from 38 centres were analysed. The numbers of antenatal patients seen yearly (table 1) varied from 2000 to 14 000 (mean 4320). The percentages and numbers of patients screened are shown in table 2. In eight centres screening for variant haemoglobins was universal and in 30 it was selective on the basis of ethnic origin. Twenty three of 38 centres requested information on ethnic origin on request forms. Only 16 of the 30 centres practising selective screening requested such information. Even when information was requested it was not necessarily provided: only four centres carrying out selective screening had ethnic information on request forms from more than 80% of patients (table 3).

Table 2 Percentage of antenatal patients screened in relation to total number of antenatal patients

Screening tests per year as a percentage of total antenatal patients	*Number of centres*	*Total number of antenatal patients per year (range)*
¾–10	10	128–630
11–20	5	450–1600
21–30	2	650–1000
31–40		
41–50	4	1500–2600
51–60	2	2400–2500
61–70	1	4500
71–80		
81–90	1	3000
91–100	7	2000–6000

Table 3 Answers to the question "How fully is information on ethnic origin completed on request forms?" for those carrying out selective or universal screening

Information complete	*Selective*	*Universal*
¾–20%	5	
21–40%	2	
41–60%	1	
61–80%	2	3
81–100%	4	1
Not stated	4	1
Total	18	5

SCREENING FOR β THALASSAEMIA TRAIT

All laboratories sought to diagnose all cases of β thalassaemia trait. Five centres measured the percentage of haemoglobin A_2 on all antenatal patients and were thus testing for β thalassaemia trait regardless of red cell indices or ethnic origin. Twenty four centres were screening (that is, selecting patients for further investigation) on the basis of red cell indices alone, while nine laboratories were selecting for testing on the basis of both ethnic origin and red cell indices. Those screening on the basis of red cell indices used the mean cell volume (MCV) alone in six cases, mean cell haemoglobin (MCH) alone in six cases, and both indices in 20 cases. The cut off points selected for screening varied from an MCV of < 65 fl to an MCV of < 80 fl and, similarly, from an MCH of < 26 pg to an MCH of < 27.1 pg. There was a similar variation in cut off points when both the MCV and the MCH were used. It was estimated that the cut off points selected would lead to seven of the 32 laboratories missing more than 2% of cases of β thalassaemia trait. Of these seven laboratories, six could have missed between 2% and 10% of cases, while the laboratory using a cut off point of < 65 fl could miss up to 30% of cases. There was not always an adequate appreciation of the importance of technical details in determining suitable cut off points for screening. For example, some centres using Coulter counters did not know whether the instrument was calibrated with or without a plasma trapping correction. Some of the factors known to influence the MCV measurement are shown in table 4. Fewer factors affect estimates of the MCH, since cell shrinkage and an arbitrary plasma trapping correction do not affect this calculated variable.

Twenty nine laboratories screened for β thalassaemia trait regardless of ethnic group (five had universal testing, 29 selected on the basis of red cell indices). Of the nine laboratories which selected on the basis of ethnic group, the

Table 4 Technical factors affecting measurements of mean cell volume (MCV)

Factor	*Effect*
Salt of EDTA used as anticoagulant	K_3EDTA causes cells to shrink about 2% in comparison with cells taken into K_2EDTA so packed cell volume (PCV) and MCV are about 3% lower[3]
Liquid rather than dry EDTA	Dilution effect, 0.5% reduction in PCV/haematocrit
Arbitrary 3% correction for plasma trapping (applied in some laboratories using Coulter counters)	Measured PCV and MCV 3% lower so that a cut off point of 80 fl with a plasma trapping correction would be approximately equivalent to a cut off point of 83 fl without a plasma trapping correction*
Use of a control material as a calibrant	MCV may vary by several fl so that instruments may be miscalibrated to this extent
Swelling of red cells on storage at room temperature (as measured by Technicon H.1 series instruments)	Average rise of 4 fl by 24 hours[4]

*Quite fortuitously the PCV of a sample taken into liquid K_3EDTA with the PCV measured without a plasma trapping correction is likely to be very similar to the PCV of the same blood specimen taken into dry K_2EDTA with a plasma trapping correction being applied to the PCV3.

selection was made by the antenatal clinic in two cases, by a combination of the antenatal clinic and the laboratory in five cases, and by the laboratory in two cases. In only one instance was ethnic information available to the laboratory on more than 80% of women. In the two hospitals where the laboratory was making the selection of women to be tested there was information on ethnic group in less than 5% of women. One of these laboratories was using the patient's name to try to identify women who were not northern European.

SCREENING FOR α^0 THALASSAEMIA TRAIT

Only two thirds of laboratories stated that they sought to diagnose all cases of α^0 thalassaemia trait, that is, all women with the $--/\alpha\alpha$ genotype who, if the partner were similarly affected, would be at risk of producing a fetus with haemoglobin Barts hydrops fetalis. Although all laboratories specified that they tested all South East Asians ± Chinese there was otherwise no consensus as to which ethnic groups should be tested (table 5). Women of the designated ethnic groups were screened on the basis of MCV alone (6), MCH alone (12) or MCV plus MCH (14). Six laboratories did not state their criteria or were in the process of determining policy. As for β thalassaemia trait, the actual cut off points were very variable. MCV criteria ranged from < 75 fl to < 80 fl and MCH criteria from < 23 pg to < 27 pg. Criteria when both MCV and MCH were used were similarly variable. If the BCSH guideline is accepted, that is, that an MCH of < 26 pg is used as a criterion for further testing,[2] then

Table 5 Ethnic groups other than South East Asian and Chinese screened for α^0 thalassaemia trait

Ethnic groups screened (as described by the respondent)	*Number of laboratories*
South-East Asian ± Chinese only	5
Mediterranean	6
Cypriot	2
Cypriot, Greek, and Turkish	1
Mediterranean and Middle-Eastern	3
Mediterranean and British	1
All non-northern European	3
All who are not northern European, African, or South Asian	1
Other	3
Universal	12

Table 6 Answers to the question "Whom do you screen for sickle cell trait?"

Ethnic criteria (as described by the respondent)	*Number of laboratories*
Afro-Caribbean	1
Africans, Afro-Caribbean	8
Africans, Afro-Caribbean, and antenatal clinic referrals	1
Africans, Afro-Caribbean, Bangladeshis	1
Africans, Afro-Caribbean, South Asians, Mediterranean	2
All non-northern Europeans	14
Non-northern Europeans or partner non-northern European	1
Universal screening	8
Antenatal staff select referrals	2

seven of 38 laboratories may be missing cases while 15 of 38 may be performing unnecessary testing and causing unwarranted anxiety. However, it should be noted that the BCSH criteria are conservative, selected to try to avoid missing any cases, and it is probable that the great majority of people with α^0 thalassaemia trait have an MCH of less than 25 pg. If this is so then only three laboratories are likely to be missing a significant number of cases.

TESTING FOR VARIANT HAEMOGLOBINS

Thirty seven of 38 laboratories stated that they sought to detect all women with haemoglobin S. Despite this only 23 laboratories screened all the relevant ethnic groups (table 6). Thirty seven laboratories sought to detect all women with haemoglobin C and 36 all those with haemoglobins E or D. Six mentioned that they sought to detect haemoglobin O Arab.

TESTING OF PARTNERS

All laboratories sought to test the partners of women with β thalassaemia trait for both β thalassaemia trait and haemoglobin S, and all but one tested partners for haemoglobin E. All laboratories also sought to test the partners of women with haemoglobin S for haemoglobins S and C and for β thalassaemia trait. However, only 10 aimed to test the partners of women with haemoglobin S for haemoglobin D, six for haemoglobin O Arab, and three for haemoglobin E. Although these haemoglobins would be detected if haemoglobin electrophoresis were carried out, the fact that there was not a policy aimed at their detection might mean that not all partners of appropriate ethnic groups were screened. Although we did not ask specifically, it is likely that all laboratories that sought to diagnose α^0 thalassaemia trait also sought to detect α^0 thalassaemia trait in the partners of affected women. Most laboratories sought to screen the partner by determining the red cell indices once α^0 thalassaemia trait was suspected in the woman (that is, when appropriate indices with a normal haemoglobin A_2 were identified), thus saving unnecessary laboratory work if the partner clearly did not have α^0 thalassaemia trait.

CHOICE OF TECHNICAL METHODS

Twenty nine laboratories gave information on their method for measuring haemoglobin A_2 for the diagnosis of β thalassaemia trait. Ten used high performance liquid chromatography

(HPLC) and 15 used microcolumn chromatography, both of these being satisfactory methods. One laboratory used electrophoresis with elution. This is also a satisfactory method, although it is rather labour intensive and therefore may be less suitable for large numbers. One laboratory used densitometry, which is not regarded as a sufficiently precise method to be appropriate for this purpose. Another used isoelectric focusing which has not yet been validated for this purpose.

Thirty seven laboratories gave information on their methods for diagnosis of α^0 thalassaemia trait. Twenty four laboratories tested for haemoglobin H inclusions. Six laboratories proceeded to DNA analysis only if H inclusions were detected, while eight laboratories performed DNA analysis regardless of the results of H inclusions. Nine laboratories which looked for H inclusions used DNA analysis in a selective manner but the basis of this selection was not stated. Ten laboratories used DNA analysis as the initial diagnostic method and did not examine for H inclusions. Overall DNA analysis, which is the definitive method for the diagnosis of α^0 thalassaemia trait, was used by 33 laboratories but four apparently did not perform or arrange this test.

Most laboratories used a wide range of methods for the detection and identification of variant haemoglobins, including electrophoresis on cellulose acetate at alkaline pH (35 laboratories), electrophoresis on citrate agar or agarose at acid pH (28), high performance liquid chromatography (19), and isoelectric focusing (13). All laboratories used at least two methods for the identification of haemoglobin S, and 34 laboratories used at least two methods for the identification of other variant haemoglobins.

PROBLEMS EXPERIENCED

Participants were asked to list the problems they experienced in providing this diagnostic service. Results are shown in table 7.

Table 7 Problems encountered with antenatal screening

Category	*Nature*	*Number*	*Total in category*
Patients/partners	Delayed booking/testing	10	
	Unavailable partner/delayed partner testing	15	25
Inadequate funds	To permit universal screening	7	
	To train antenatal staff	2	
	For DNA analysis	1	
	For funding specific laboratory	1	
	For counsellors	4	
	For laboratory staff	3	18
Laboratory	Diagnosis of α thalassaemia trait	10	
	Diagnosis of β thalassaemia trait with normal haemoglobin A_2	4	14
Application of protocols	Identifying "at risk" pregnancies	6	
	Testing the baby	2	
	Testing the family	3	11
Communication	Obtaining appropriate sample from patient	1	
	Community based antenatal care	1	
	Cooperation of obstetricians	1	
	Communication with antenatal clinic	1	
	No screening protocol	1	
	Education of midwives	2	7
Data collection	Ethnic origin	3	
	Data on patient	2	
	Patients without haemoglobinopathy cards	1	
	Inadequate audit	1	7

Discussion

An adequate screening programme for inherited disorders of globin genes should predict the great majority of pregnancies at risk of β thalassaemia major, haemoglobin Barts hydrops fetalis, and sickling disorders (SS, SC, S/DPunjab, S/OArab) or haemoglobin E/β thalassaemia. This necessitates either universal screening of antenatal patients, with clear implications with regard to cost, or selective screening of those at significant risk. Selective screening could be initiated either by antenatal clinic staff or by laboratory staff. The genetics of these disorders is complex, the ethnic groups in which they occur are diverse, and the likely clinical severity is not always easy to predict. For these reasons, although antenatal clinic staff should be able to identify women who are not of northern European origin, it is unlikely that they would be able to initiate selective screening unless they are following very clear and detailed guidelines prepared in conjunction with haematology staff. If selective screening is to be initiated by laboratory staff it is essential that they have detailed and accurate information on ethnic origin on the great majority of women. It is clear that at present this information is not available in an acceptable proportion of patients.

Some laboratories have a sufficiently high percentage of patients drawn from ethnic minorities that universal testing for β thalassaemia trait can be carried out, usually by measuring haemoglobin A_2 by HPLC on all women. The majority of laboratories still seek to screen initially on the basis of red cell indices and apply haemoglobin A_2 measurements selectively. If the latter policy is followed it is important that the cut off point selected should permit the diagnosis of the great majority of cases, for example $\geq 98\%$. The MCH has some theoretical advantages over the MCV for screening purposes since the technical factors affecting its measurement are fewer. It is the method recommended by the SMAC report.[1] For laboratories using Technicon counters and where there is an appreciable delay in receiving specimens the instability of the measured MCV means that use of the MCH is certainly preferable. For impedance counters (such as Coulter and Sysmex instruments) either the MCV or the MCH can be used as long as the cut off point is appropriate, having been selected after consideration of all the relevant technical factors. Ideally laboratories should determine their own reference ranges and establish cut off points for their own techniques. However, this is very labour intensive and usually takes several years. An alternative is to accept cut off points established in a laboratory using exactly the same techniques. When a woman is found to have β thalassaemia trait her partner should be screened for β thalassaemia trait, haemoglobin S, and haemoglobin E.

It is of some concern that only two thirds of laboratories sought to detect all women with α^0 thalassaemia trait. It is desirable that the great majority of such cases should be diagnosed, at least in those ethnic groups where there is a reasonable probability of the partner also

having α^0 thalassaemia trait. There is general agreement that all women of South East Asian and Chinese ethnic origin should be screened. Other groups who could reasonably be considered for testing are Cypriots, Greeks, and Turks.[5] It is not generally considered necessary to test African or Afro-Caribbean women or those from the Indian subcontinent ("South Asians") since, although these groups have a significant prevalence of α^+ thalassaemia trait or homozygosity (genotype $-\alpha/\alpha\alpha$ or $-\alpha/-\alpha$) they are not at significant risk of α^0 thalassaemia trait or of having a fetus with haemoglobin Barts hydrops fetalis. It is also not generally considered necessary to test white British women since although α^0 thalassaemia trait does occur in this ethnic group it is rare, so unless the partner were Chinese or South East Asian or of another relevant ethnic group hydrops fetalis would be extremely unlikely. In testing for α^0 thalassaemia trait it is necessary to keep in mind not only the cost of the test but the inevitable delay in obtaining results and the amount of anxiety which is generated when this test is performed unnecessarily. When a woman is found to have α^0 thalassaemia trait her partner should be screened by determining red cell indices. To avoid unnecessary and expensive testing and anxiety in the potential parents, it is reasonable to determine the red cell indices on the partner as soon as α^0 thalassaemia trait is suspected and if his MCH is 26 pg or higher no further testing is needed in either partner. It should be emphasised that it is appropriate to have different cut off points for the diagnosis of β thalassaemia trait and for α^0 thalassaemia trait (for example, < 27 pg for β thalassaemia trait and < 26 pg, or possibly less than 25 pg, for α^0 thalassaemia).

It is desirable to predict the great majority of pregnancies at risk of sickle cell anaemia (SS) and the compound heterozygous states S/β thalassaemia, SC, S/DPunjab, and S/OArab. E/β thalassaemia should also be predicted since it causes a thalassaemic disorder of variable severity but sometimes necessitating regular blood transfusions. It is therefore desirable to test all relevant ethnic groups for haemoglobins S, C, D Punjab, and O Arab in addition to testing for β thalassaemia trait. In effect this necessitates testing all those who are not of northern European ancestry. For example, haemoglobin S occurs not only in those with African ancestry but also in the Indian subcontinent and in Arabs, Greeks, Cypriots, and Italians. Once there is a significant proportion of ethnic minority women (the SMAC report suggest more than 15%)[1] it becomes administratively much easier to test all pregnant women. When any of these variant haemoglobins is detected the partner should be tested for variant haemoglobins. If the woman has haemoglobin S or E it is also important to test the partner for β thalassaemia trait.

The great majority of laboratories are using appropriate methods for the measurement of haemoglobin A_2 and for the identification of variant haemoglobins. There is no unanimity of opinion with regard to testing for α^0 thalassaemia trait. For example the place of testing for haemoglobin H inclusions is not clear. If there is a clear indication for testing for α^0 thalassaemia trait then it may be cost–effective to proceed directly to DNA analysis. Conversely, if there is no good reason to suspect α^0 thalassaemia trait then it may be more appropriate not to test at all.

Laboratories experience many problems in seeking to provide an appropriate diagnostic service for the detection of globin gene abnormalities in antenatal patients. Among these should be noted: problems caused by late booking of women at antenatal clinics; unavailability of the partner for testing; lack of adequate information on ethnic origin; and difficulties in communication between the different professional groups involved. Economic pressures have an influence on many parts of the service. In addition to these logistic and financial constraints, there are still some technical problems to be resolved. The costs and inherent delays in DNA analysis for diagnosis of α^0 thalassaemia mean that it is harder to establish adequate protocols for diagnosis of this condition than for β thalassaemia trait and variant haemoglobins. The place, if any, of testing for haemoglobin H inclusions is not clear. There are some other problem areas which we have not considered such as the diagnosis of silent β thalassaemia trait and the diagnosis of coinheritance of β and δ thalassaemia. However, there is still room for improvement in the development of policies for the diagnosis of the more common straightforward cases. There would appear to be a place for more detailed guidelines in this field in order to ensure the appropriate use of tests which will identify the great majority of significant abnormalities in a cost–effective manner and without causing unnecessary anxiety to potential parents. A haemoglobinopathy working party of the BCSH is now drawing up such guidelines.

We wish to acknowledge the assistance of Professor Bernadette Modell, previously Honorary Secretary of the UK Forum, and to thank all members of the Forum for their collaboration.

Appendix 1

QUESTIONNAIRE

1. Name and address of hospital.....
2. Name/designation of person completing form .....
3. Number of women attending antenatal service in a year .....
4. Number of requests for antenatal haemoglobinopathy screening per year .....

5. With regard to antenatal screening for thalassaemias and haemoglobinopathies:
   - Do you have a written policy, e.g., a standard operating procedure? YES/NO
   - Do you request information on ethnic origin on request forms for antenatal patients? YES/NO
   - Is this information usually completed on these forms? –Please estimate percentage completed .....%
   - With regard to screening for abnormal haemoglobins
     –Is your screening SELECTIVE/UNIVERSAL
   - If selective, by whom are decisions made as to whether individual women require screening?
     –antenatal clinic staff YES/NO
     –haematology laboratory staff YES/NO
     –other (please specify) .....

6. With regard to screening for β thalassaemia trait:
   - Do you screen by the MCV? YES/NO
   - If you screen by the MCV what is the level below which you measure the haemoglobin A_2 percentage? .....
   - Do you screen by the MCH? YES/NO
   - If you screen by the MCH what is the level below which you measure the haemoglobin A_2 percentage? .....
   - What instrument do you use for antenatal blood counts? .....
   - Do you use a plasma trapping correction in calibrating your instrument? YES/NO/DON'T KNOW
   - With regard to haemoglobin A_2 estimation is this done on all samples with an MCV/MCH below your cut off point or is it selective, i.e., limited to certain ethnic groups? ALL/SELECTIVE
   - If selective, which ethnic groups are tested? .....
   - If screening is selective who makes the decision as to who should be tested? –antenatal clinic staff YES/NO –haematology laboratory staff YES/NO –other (please specify).....
   - What technique do you use for measuring haemoglobin A_2? Please specify .....

7. With regard to screening for α^0 thalassaemia trait:
   - If there is microcytosis and the haemoglobin A_2 is normal or low, whom do you test for α^0 thalassaemia trait? CERTAIN ETHNIC GROUPS/UNIVERSAL
   - If screening is selective, which ethnic groups are screened? .....
   - If screening is selective, by whom are decisions made as to which individual women require screening?
     –antenatal clinic staff YES/NO
     –haematology laboratory staff YES/NO
     –other (please specify).....
   - Do you screen by the MCV? YES/NO
   - If you screen by the MCV what is the cut off point below which you carry out further testing?
     .....
   - Do you screen by the MCH? YES/NO
   - If you screen by the MCH what is the cut off point below which you carry out further testing?
     .....
   - If your MCV/MCH criteria are met, do you screen by:
     –looking for haemoglobin H inclusions? YES/NO
     –DNA analysis only if haemoglobin H inclusions are detected? YES/NO
     –only DNA analysis? YES/NO
   - At what point so you send for the partner? .....
   - If the woman is a carrier of α^0 thalassaemia trait do you recommend screening: REGARDLESS OF ETHNIC GROUP/IN SELECTED ETHNIC GROUPS
   - If in selected ethnic groups please specify which .....

8. With regard to detection of women carrying the sickle cell gene do you test:
   - All Africans YES/NO
   - All sub-Saharan Africans YES/NO
   - All Afro-Caribbeans/West Indians YES/NO
   - Other ethnic groups (please specify) .....

9. Which cases of the following abnormalities do you aim to detect in your antenatal screening programme?
   - Haemoglobin S ALL/SOME/NONE
   - Haemoglobin C ALL/SOME/NONE
   - β Thalassaemia trait ALL/SOME/NONE
   - α^0 Thalassaemia trait ALL/SOME/NONE
   - Haemoglobin E ALL/SOME/NONE
   - Haemoglobin D Punjab ALL/SOME/NONE
   - Other (please specify) .....
10. What techniques do you use for screening for variant haemoglobins? (please circle any techniques used)
   - Sickle solubility test
   - Haemoglobin electrophoresis on cellulose acetate at alkaline pH
   - Haemoglobin electrophoresis on citrate agar/agarose gel at acid pH
   - High performance liquid chromatography (HPLC)
   - Isoelectric focusing (IEF)
   - Other (please specify) .....
11. If you detect haemoglobin S in an antenatal patient which abnormalities do you seek to detect in the partner?
   - Haemoglobin S YES/NO
   - Haemoglobin C YES/NO
   - β Thalassaemia trait YES/NO
   - Other (please specify) .....
12. If you detect β thalassaemia trait in an antenatal patient which abnormalities do you seek to detect in the partner?
   - β Thalassaemia trait YES/NO
   - Haemoglobin S YES/NO
   - Haemoglobin E YES/NO
   - Other (please specify) .....
13. Do you follow any specific published guidelines in your antenatal screening programme? YES/NO
   If yes, please specify .....
14. If a patient comes to the antenatal clinic with a haemoglobinopathy card which has been issued by your own or another laboratory do you:
   ACCEPT THE DIAGNOSIS/RETEST
15. What would you say are the main problems in antenatal screening for disorders of globin chain synthesis? .....
16. At what point do you do a serum iron or ferritin assay? .....

Appendix 2

HOSPITALS RETURNING QUESTIONNAIRES

Birmingham: City Hospital
Cardiff: University Hospital of Wales
Derby: Derby City Hospital
Halifax: Halifax General Hospital
Glasgow: Royal Hospital for Sick Children and Queen Mother's Hospital
Gloucester: Gloucester Royal NHS Trust
Greenwich: Greenwich District Hospital
High Wycombe: Wycombe General Hospital
Leicester: Leicester Royal Infirmary
Liverpool: Alder Hey Children's Hospital (no antenatal practice)
London: Central Middlesex Hospital; Chase Farm Hospital; Charing Cross Hospital; Chelsea and Westminster Hospital; Guys and St Thomas's Trust; Hammersmith Hospital; Hillingdon Hospital; Homerton Hospital; Hospital for Sick Children (no antenatal practice); King's College Hospital; Royal Free Hospital; Royal London Hospital; St Mary's Hospital; Newham General Hospital; North Middlesex Hospital; Wellhouse NHS Trust (Barnet General Hospital); Whipps Cross Hospital; Whittington Hospital
Luton: Luton and Dunstable Hospital
Manchester: Manchester Royal Infirmary

Middleborough: Middleborough General Hospital
Nottingham: City Hospital; Queen's Medical Centre
Newcastle Upon Tyne: Royal Victoria Infirmary
Orpington: Bromley Hospitals NHS Trust (Farnborough Hospital)
Reading: Royal Berkshire Hospital
Sheffield: Central Sheffield University Hospitals (with Royal Hallamshire and Northern General)
Sidcup: Queen Mary's Hospital
Slough: Wexham Park Hospital
Walsall: Manor Hospital
Welwyn Garden City: East Herts NHS Hospital Trust (QEII Hospital)

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