GUIDELINE

Significant haemoglobinopathies: A guideline for screening and diagnosis

A British Society for Haematology Guideline

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Summary
Antenatal screening/testing of pregnant women should be carried out according to the guidelines of the National Health Service (NHS) Sickle Cell and Thalassaemia Screening Programme. Newborn screening and, when necessary, follow-up testing and referral, should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening Programme. All babies under 1 year of age arriving in the United Kingdom should be offered screening for sickle cell disease (SCD). Preoperative screening for SCD should be carried out in patients from ethnic groups in which there is a significant prevalence of the condition. Emergency screening with a sickle solubility test must always be followed by definitive analysis. Laboratories performing antenatal screening should utilise methods that are capable of detecting significant variants and are capable of quantitating haemoglobins A2 and F at the cut-off points required by the national antenatal screening programme. The laboratory must ensure a provisional report is available for antenatal patients within three working days from sample receipt.

Keywords
genetic disorders, haemoglobinopathies, laboratory haematology, sickle cell anaemia, sickle cell disease, thalassaemia

OBJECTIVES

Disorders of globin chain synthesis, both thalassaemia and haemoglobin variants, are common in the United Kingdom and constitute a significant public health problem. Diagnosis may be required: (i) to confirm a provisional clinical diagnosis, such as sickle cell disease (SCD) or β thalassaemia major (now often referred to as transfusion-dependent thalassaemia or TDT); (ii) to explain a haematological abnormality, such as anaemia or microcytosis; (iii) to identify an abnormality in the presymptomatic phase, as in neonatal screening; (iv) to identify foetuses at risk of significant haemoglobinopathies and offer the parents informed choice; (v) to permit genetic counselling of prospective parents; (vi) to identify the presence of sickle cell haemoglobin preoperatively. Improved fully automated systems and reagents for techniques such as cation-exchange high-performance liquid chromatography (HPLC) and capillary electrophoresis

Abbreviations: CAE, cellulose acetate electrophoresis; CE, capillary electrophoresis; EQA, External Quality Assessment; FBC, full blood count; FOQ, Family Origin Questionnaire; Hb, haemoglobin; HPFH, hereditary persistence of fetal haemoglobin; HPLC, high-performance liquid chromatography; IEF, isoelectric focusing; MCH, mean cell haemoglobin; MCV, mean cell volume; MS, mass spectrometry; NTD, non-transfusion-dependent thalassaemia; NHS, National Health Service; NSC, National Screening Committee; PHE, Public Health England; PND, prenatal diagnosis; SCD, sickle cell disease; TDT, transfusion-dependent thalassaemia.
Haemoglobinopathies: Screening and Diagnosis

(CE) have led to their general introduction. There is also increasing use of other methods to identify globin gene abnormalities including DNA analysis, mass spectrometry and immunological methods. There is, therefore, a need for an updated guideline defining the role of new techniques and their place in screening and in specific diagnostic settings. The detection of unstable haemoglobins, methaemoglobins and high- and low-oxygen affinity haemoglobins is not discussed but laboratories should either have methods for detecting these variant haemoglobins or should refer such samples to a reference laboratory.

Investigation of haemoglobinopathies follows two different approaches, screening and diagnosis. Screening operates at a national level with designated policies, algorithms and risk assessments, with the aim of achieving maximum benefit whilst minimising false positive and false negative results. Definitive diagnosis requires more thorough investigation, increasingly using molecular techniques to improve accuracy. Unless molecular or specialised mass spectrometry techniques have been used, the identification of haemoglobins is often presumptive, based on migration patterns or other characteristics in an individual of appropriate family origin. Presumptive identification must be based on a minimum of two techniques based on different but complementary principles. Family studies can also be of considerable importance in elucidating the nature of disorders of haemoglobin synthesis. As testing for haemoglobinopathies has implications for genetic counselling, informed consent should be obtained from individuals prior to screening.

Throughout these guidelines, the term ‘sickle cell disease’ (SCD) encompasses both homozygous and the compound heterozygous states that lead to symptomatic disease as the result of the presence of haemoglobin S. Sickle cell anaemia refers specifically to those homozygous for $\beta^S$. These guidelines are intended for UK Haematologists and Biomedical and Clinical Scientists and the approach to screening is that which is considered practical and feasible for the British population. Different strategies may be required for populations with a different prevalence of haemoglobinopathies.

METHODS

These guidelines are an update of previous guidelines and were written by clinical and laboratory experts representing areas with a high and low prevalence of haemoglobin disorders. Sections relating to antenatal and newborn screening are based on guidelines published by the National Health Service (NHS) Sickle Cell and Thalassaemia Screening Programme and available in the programme’s Laboratory Handbooks.

For this updated guideline, PubMed, MEDLINE and EMBASE were searched systematically for publications in English from January 2015 to June 2021 using keywords (see Appendix A). The guideline was compiled according to the British Society for Haematology process. The writing group produced a draft guideline, which was subsequently reviewed by consensus by members of the General Haematology Task Force of the British Society for Haematology. The guideline was then reviewed by a sounding board of UK haematologists and the British Society for Haematology Committee and comments were incorporated as appropriate. Criteria used to quote levels and grades of evidence are as outlined in https://www.gradeworkinggroup.org/.

Introduction

Haemoglobin synthesis during development

The normal pattern of haemoglobin synthesis during embryonic, foetal and adult life is summarised in Figure 1. Foetal haemoglobin, haemoglobin (Hb) $F$, ($\alpha_2\gamma_2$) represents 90%–95% of all haemoglobin by 34–36 weeks gestation. Until about 34 weeks gestation, adult haemoglobin, Hb A ($\alpha_2\beta_2$), accounts for 4%–13% of total haemoglobin in the foetus. After 34 weeks gestation, Hb A production increases significantly as Hb F production falls. At term, Hb F represents 5%–9% of all haemoglobin with Hb A levels reaching, on average, 20%–30%. In addition to being increased in some haemoglobinopathies, an increased percentage of Hb F can be seen in infants who are small for gestational age, who have experienced chronic hypoxia or who have trisomy 13 as well as in the benign condition, hereditary persistence of foetal haemoglobin (HPFH).

Haemoglobin F percentage remains static for the first 2 weeks of life and then decreases by approximately 3% per week when erythropoiesis recommences and is normally <2%–3% of total haemoglobin by 6 months of age. Hb A becomes the predominant haemoglobin by 3 months of age, although this switch may be delayed in sick preterm infants. Haemoglobin $A_2$ ($\alpha_2\beta_2$) is produced in small amounts from birth and usually reaches adult levels by 6 months of age.

![Figure 1: Expression of different haemoglobins during normal development (reproduced from Bain with permission of Wiley-Blackwell).](https://onlinelibrary.wiley.com/doi/abs/10.1111/bj.13652141)
Indications for screening and testing

Preconceptual diagnosis of haemoglobinopathies is recommended in at-risk groups (Table 1). This can be important because it can be difficult to complete antenatal screening and foetal diagnosis within the first 12 weeks of pregnancy if the couple is unaware of the risk and it also opens the possibility of preimplantation genetic diagnosis. This is most likely to be feasible in general practice but other medical practitioners should be alert to the possibility of a carrier state for a haemoglobinopathy and should offer screening. In some circumstances, premarital screening may be preferred. The individuals concerned must be informed of the result, whether or not an abnormality is found. If a relevant abnormality is detected (Hb variant or possible/probable thalassaemia), partner screening should be offered, according to the antenatal screening algorithm (see below), if appropriate.

Recommendations

- General practitioners should consider preconceptual screening and diagnosis of their patients of childbearing age in high-risk groups. (1A)
- General practitioners should consider and discuss premarital screening and diagnosis of patients of childbearing age in appropriate circumstances. (1A)
- Clinics carrying out assisted conception should always carry out relevant screening and, when necessary, testing of both the egg donor and/or the sperm donor. (1A)

Antenatal screening

National Screening Committee policy recommends antenatal screening for SCD and thalassaemia. Clinically significant haemoglobinopathies that should be detected are shown in Table 2. The recommended procedure differs according to whether the antenatal unit is in a high or low-prevalence area for SCD and thalassaemia. For high-prevalence areas (2% or more of the antenatal booking bloods are screen positive, that is are positive for any of the conditions shown in

<table>
<thead>
<tr>
<th>Condition to be detected</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Significant maternal haemoglobinopathies</td>
<td>SS and other types of sickle cell disease (Hb SC, Hb S/β thalassaemia, etc.)</td>
</tr>
<tr>
<td>(ii) Maternal conditions requiring testing of the baby’s biological father</td>
<td>Significant disorders to be detected in the foetus</td>
</tr>
<tr>
<td>(a) Conditions in (i)</td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>SS or S/β thalassaemia</td>
</tr>
<tr>
<td>AC</td>
<td>SC</td>
</tr>
<tr>
<td>A/D-Punjab</td>
<td>S/D-Punjab</td>
</tr>
<tr>
<td>AE</td>
<td>SE and E/β thalassaemia</td>
</tr>
<tr>
<td>A/O-Arab</td>
<td>S/O-Arab</td>
</tr>
<tr>
<td>A/Lepore</td>
<td>S/Lepore and Lepore/β thalassaemia</td>
</tr>
<tr>
<td>δβ thalassaemia trait</td>
<td>S/β thalassaemia and δβ thalassaemia homozygosity or compound heterozygosity</td>
</tr>
<tr>
<td>6α thalassaemia trait</td>
<td>S/δβ thalassaemia and 6δ thalassaemia homozygosity or compound heterozygosity with δ thalassaemia</td>
</tr>
<tr>
<td>HFPFH</td>
<td>S/HFPFH*</td>
</tr>
<tr>
<td>(c) Any compound heterozygous state including one or more of the above conditions</td>
<td></td>
</tr>
<tr>
<td>(d) Any homozygous state of the above conditions</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Hb, haemoglobin; HFPFH, hereditary persistence of foetal haemoglobin; NTDT, non-transfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia.

*While this is a mild condition clinically, it may cause diagnostic confusion in the neonate.
Table 2), universal laboratory screening and use of the Family Origin Questionnaire (FOQ) (Appendix B) is advised. For low-prevalence areas (less than 1% of the antenatal booking bloods are screen positive), screening is based on assessing the individual risk by determining the family origin of the woman and of the biological father of the baby by means of the FOQ. Units with a prevalence between these two values should continue to use their current algorithm and regard that for the high-prevalence areas as the gold standard. Whichever screening method is applied, the laboratory must ensure a provisional report is available within three working days from sample receipt.

Screening in high-prevalence areas
The screening policy for high-prevalence areas starts with a full blood count (FBC) and HPLC, CE or suitable alternative technique on a maternal blood sample. An FOQ is completed in every pregnancy and is sent to the laboratory with the sample. Consent for testing must be obtained and recorded. Testing of the women should ideally be completed before 10 weeks and the whole process including testing of the baby’s biological father, if applicable, should be completed within the first 12 weeks of pregnancy; even women presenting for the first time late in pregnancy should be offered testing because the results will be relevant both to this and future pregnancies and will enable genetic advice about future pregnancies to be given.

Screening in low-prevalence areas
For low-prevalence areas it is recommended that all women be offered appropriate screening with the FOQ used to determine the family origins of the mother and the baby’s biological father and thus the specific tests to be employed. All should have an FBC and the red cell indices should be assessed and acted on in a similar manner as for high-prevalence areas. Haemoglobin analysis is otherwise confined to those women whose own or the baby’s biological father’s family origins are not Northern European or are unknown.

Recommendation

Antenatal screening/testing of pregnant women should be carried out in accordance with the guidelines of the NHS Sickle Cell and Thalassaemia Screening Programme. (1A)

Assessment of abnormal antenatal screening results

Detection of a haemoglobin variant
If a significant Hb variant is identified it should be confirmed by a suitable alternative method (e.g. haemoglobin electrophoresis, CE or isoelectric focusing [IEF] if the initial method was HPLC) and the baby’s biological father should be offered screening without waiting for the definitive result on the mother. Variant haemoglobins of clinical relevance in this context are haemoglobins S, C, D-Punjab, E, H, Lepore and O-Arab.

Raised Hb A2 percentage
If no relevant variant haemoglobin is identified, Hb A2 percentage should be assessed, when appropriate. This is essential if the mean cell haemoglobin (MCH) is <27 pg. An Hb A2 level of ≥3.5% in the presence of a MCH<27 pg indicates heterozygosity for β thalassaemia. An Hb A2 of >4% with a normal MCH should be assessed further as it may indicate a milder β thalassaemia carrier state that would warrant testing of the baby’s biological father (see below). It is recognised that in some mild or atypical β mutations, the Hb A2 levels may fall below 3.5%. This is something that is covered in the UK programme’s risk assessment and monitored via incidents/outcome data and in the UK population an action value of 3.5% is effective. In addition, co-existing delta thalassaemia may reduce the Hb A2 into the normal range and mask a beta thalassaemia trait. If the Hb A2 is apparently 8% or higher on HPLC, a diagnosis of Hb Lepore should be considered, whereas an Hb A2 level apparently >15% may indicate Hb E trait. Other variant haemoglobins also have a retention time similar to that of Hb A2 on HPLC. The laboratory should be alert to the presence of an Hb A2 variant necessitating the summing of HbA2 and the variant. It should be noted that using HPLC the Hb A2 variant is not calibrated, and therefore, the total of the combined peaks is an estimate which can be considered sufficient for screening purposes.

Raised Hb F percentage
In the context of an MCH <27 pg, an isolated raised Hb F of ≥5% identifies possible heterozygosity for δβ thalassaemia and testing of the baby’s biological father is required. In the presence of a normal MCH, HPFH should be considered when the Hb F is ≥10%.

Possible α thalassaemia heterozygosity
In the absence of a variant Hb and β or δβ thalassaemia heterozygosity, α thalassaemia carrier states should be considered if the MCH is <27 pg. This should be considered regardless of iron status as there is insufficient time in the antenatal setting to reassess indices after iron treatment. If the MCH is <25 pg, the individual should be assessed for the possibility of α thalassaemia in the light of his or her family origin. Family origins that indicate that α thalassaemia is likely are shown in Table 1. An alternative explanation for these laboratory findings is homozygosity for α thalassaemia or iron deficiency, which may also be seen in these ethnic groups.

α thalassaemia is found in many ethnic groups, with a high (10%–30%) carrier frequency in some parts of Africa and Southeast Asia. Even if both parents are carriers, there is no risk to the foetus. Homozygous α thalassaemia is not a clinically significant disorder with respect to genetic or obstetric complications but can cause diagnostic confusion with αδ thalassaemia trait or iron deficiency.
Heterozygotes (carriers) generally have a MCH of 25–28 pg and a normal Hb A2 level. Approximately one-third of cases are silent.

Homozgyotes generally have a MCH<25 pg, as seen in carriers for α0 thalassaemia.

If the MCH is <25 pg and α0 thalassaemia is possible, the approach that makes best use of resources is to assess the family origins and red cell indices of the baby’s biological father and proceed to DNA analysis, simultaneously in the woman and the baby’s biological father, only if both are at risk of α0 thalassaemia. However, if there is any delay in obtaining a blood sample from the baby’s biological father or if he is not available, then it is appropriate to test the mother’s DNA. If the woman has haemoglobin H disease the baby’s biological father also requires assessment for α0 thalassaemia. Ultrasound examination to detect foetal anaemia may be offered as an alternative to foetal DNA testing from 12 to 16 weeks in cases where the latter is declined.

α0 thalassaemia occurs, rarely, in other ethnic groups, for example Pakistanis, Indians, some Middle Eastern populations (from United Arab Emirates, Iran, Yemen, Kuwait, Syria, Jordan) and in individuals originating in North-West England (Wigan and other parts of Lancashire). In the Middle East there is also a significant prevalence of non-deletional α thalassaemia, which can give rise to severe haemoglobin H disease in homozygotes. Similarly, in the Far East, an α thalassaemia phenotype may be the result of Hb Constant Spring or Hb Paksé. Individuals from such areas should be assessed individually, but in general, DNA analysis and partner testing is not recommended and these cases may be missed on screening. The rare cases of Hb Bart’s hydrops fetalis should be detected on ultrasound screening and DNA analysis may then be appropriate as part of the investigation for foetal anaemia.

It should be noted that a diagnosis of β thalassaemia or Hb E heterozygosity does not exclude co-existing α0 thalassaemia heterozygosity and, in ethnic groups with a significant prevalence of the latter, DNA analysis is indicated when relevant to reproductive choice. For example, if one partner has β thalassaemia or Hb E heterozygosity and the other possible α0 thalassaemia heterozygosity, both partners should be offered testing for α0 thalassaemia.

Recommendations

- The suspected presence of a significant variant of haemoglobin should be confirmed by a suitable alternative method. (1A)
- A diagnosis of β thalassaemia heterozygosity should be made on the basis of the MCH and the haemoglobin A2 percentage. (1B)
- A diagnosis of δβ thalassaemia heterozygosity should be made on the basis of the MCH and the haemoglobin F percentage. (1B)
- A diagnosis of α0 thalassaemia heterozygosity should be suspected on the basis of the MCH and a normal or low haemoglobin A2 in an individual of appropriate family origin and should be confirmed by DNA analysis of the mother and, when the same diagnosis is suspected in the biological father, by DNA analysis also in the father. (1A)
- The possibility of co-existing α0 thalassaemia heterozygosity should be assessed, on the basis of family origin, in mothers with β thalassaemia or Hb E heterozygosity and, when the diagnosis would be relevant to reproductive choice, DNA analysis should follow. (1A)

Newborn screening

National Screening Committee and Public Health England (PHE) policy is that all newborn babies should be screened for SCD. Such screening should also be extended to babies under the age of 1 year newly arrived in the United Kingdom. The major objective of the newborn screening programme is to improve outcomes in SCD through early treatment, counselling and care. The screening programme will also detect certain other variant haemoglobins by virtue of the analytical methods currently used. Additionally, the finding of Hb F only or of a very low percentage of haemoglobin A (1%–5% or less of the total haemoglobin or above the mass spectrometry action value) on the newborn screen will identify the majority of babies with β thalassaemia major/TDT, necessitating follow-up and further testing.

Neonatal screening is based on the mother’s place of residence and is done at the age of 5 days as part of the newborn dried blood spot screening programme. Further testing of samples that show a significant abnormality is required. Informed parental consent is required and, although the programme is recommended, parents have the right to opt out of testing. Opting out should be documented and the reason stated.

Clinically significant conditions that should be detected are shown in Table 3. In addition, certain conditions that are asymptomatic or have a mild phenotype will be identified and need to be subsequently distinguished from clinically significant abnormalities. For example, S/HPFH needs to be distinguished from severe forms of SCD and Hb E homozygosity needs to be distinguished from Hb E/β thalassaemia. Hb H disease may be detected but, in the United Kingdom, screening programme reporting is not recommended. Clinical follow-up, counselling and repeat testing are arranged for all babies in whom there is the possibility of a clinically significant abnormality and results are notified to the parents, general practitioner and responsible health care consultant at the place of the baby’s birth. In the case of suspected SCD, confirmatory testing and clinical follow-up should be performed in a timely manner so that penicillin prophylaxis can be started by 90 days of age; conjugate pneumococcal vaccine is now given to all babies from 12 weeks of age but is particularly important if the child has SCD.
Clinical follow-up is also necessary for all babies with Hb F only or with a low level of Hb A. Babies who have been transfused in utero or in the early neonatal period are now tested using DNA techniques for the presence of the sickle gene as part of the NHS Sickle Cell and Thalassaemia Screening Programme. If this is not available or is declined, repeat testing should be performed 4 months from the date of transfusion. Other variant haemoglobins of potential clinical or genetic significance (e.g. haemoglobins C, D-Punjab, E, O-Arab) will also be detected, and in this case, the parents, general practitioner and responsible health care practitioner at the place of the baby’s birth should be informed and parents offered counselling. Parents of babies with no significant abnormality should be informed promptly.

**Recommendations**

- All newborn babies should be screened for sickle cell disease, following the policy of the National Screening Committee and Public Health England. (1A)
- Babies in whom β thalassaemia major/TDT is suspected, on the basis of there being Hb F only or only a low concentration of Hb A, must be followed up and further testing performed. (1A)
- Detection of a significant abnormality should be followed by further testing to achieve a definitive diagnosis. (1A)
- All babies under the age of 1 year newly arrived in the United Kingdom should be screened for sickle cell disease with a significant abnormality leading to further testing to achieve a definitive diagnosis. (1A)

**General testing**

Opportunistic testing may be initiated by a general practitioner or other medical practitioners, with the informed consent of the patient, or by a haematology laboratory, where an abnormality that requires explanation is detected, for example in individuals found to have red cell indices or morphological appearances suggestive of a haemoglobinopathy or thalassaemia. Opportunistic testing for sickle cell heterozygosity is best initiated in general practice. Reflex testing by the laboratory will depend on local policy. Further testing may also be considered when a variant haemoglobin has been detected during the measurement of haemoglobin A\textsubscript{1c}.

**Recommendations**

- Haemoglobinopathy/thalassaemia testing should be initiated by general practitioners or other medical practitioners when clinically relevant and with the patient’s informed consent. (1B)
- Haematology laboratories should consider haemoglobinopathy/thalassaemia testing when laboratory results, including tests for haemoglobin A\textsubscript{1c}, indicate the possibility of a significant abnormality. (1B)

**Preoperative/preanaesthesia**

It is important to detect SCD prior to anaesthesia because its presence will influence clinical management. Testing should be initiated by clinical staff on the basis of a clinical history and assessment of family origin. All patients from groups with a high prevalence of Hb S (Table 1) should be offered testing as some cases of milder disease may be unrecognised and the presence of Hb S heterozygosity may also influence peri-operative techniques. For example, cell salvage techniques carry a risk of red cell sickling; their use is not recommended in SCD and should only be considered in sickle cell trait in a life-threatening emergency. The prolonged use of limb tourniquets should also be considered carefully in patients with sickle cell trait although there is little evidence on which to base recommendations. Appropriate counselling should be given before testing so that patients are able to give their informed consent as there may be implications for patients who discover they are carriers of the sickle cell gene. The patient/parents or guardian (for children) should be informed of the results of testing, even when negative, and the result documented in the patient’s medical record to avoid unnecessary repeat testing. Counselling should be offered if the result of the test is positive.

For routine operations, FBC and haemoglobin analysis using HPLC, CE or a suitable alternative diagnostic method should be performed at the preassessment visit. In an emergency, an FBC and a sickle solubility test should be performed. Results in this situation should be correlated with clinical features and must be followed by definitive testing (see below). It should be noted that patients with...
Hb SC and mild S/β thalassaemia may have a normal haemoglobin concentration so may be confused with sickle cell trait if clinical features and the blood film are not assessed.

**Recommendations**

- Preoperative screening for HbS should be carried out in patients from ethnic groups in which there is a significant prevalence of this variant haemoglobin. (1A)
- Emergency screening with a sickle solubility test or other screening test must always be followed by definitive analysis. (1A)

**Investigation of microcytosis outside the antenatal setting**

Patients who present for diagnosis should be distinguished from individuals (often healthy) who are being screened for haemoglobinopathies, for example in the antenatal setting. Such patients require a clinical history and physical examination and the FBC and blood film should be assessed, together with other laboratory tests, in the light of the clinical context and family origins. If there is microcytosis, appropriate tests for iron deficiency and anaemia of chronic disease should be performed and testing for thalassaemia should be considered in patients of appropriate family origin. Depending on agreed local policies, such tests may be initiated by the laboratory. Some laboratories use various published formulae to decide when to initiate such investigations but it should be noted that such formulae are not likely to be reliable in children or pregnant women or in sick patients who may have multiple medical problems influencing the haemoglobin concentration and red cell indices and their use is not recommended. Haemoglobinopathy investigations should, therefore, be considered in any unexplained microcytosis, even if the red cell indices are not typical of thalassaemia or another haemoglobinopathy.

**Recommendation**

- Patients who present for diagnosis rather than for screening, who have microcytosis, should be evaluated clinically with further testing for iron deficiency, anaemia of chronic disease and thalassaemia being carried out in the light of the red cell indices and the clinical findings. (1B)

**Laboratory investigation in the diagnosis and management of clinical disorders**

Haemoglobinopathy investigations may be indicated in the following circumstances:

- In new arrivals in the United Kingdom with a history of SCD, significant thalassaemia or unexplained anaemia.
- In patients with SCD receiving blood transfusion, including exchange transfusion, with the aim of lowering the percentage of Hb S.
- In patients with SCD or β thalassaemia intermedia, the latter now often referred to as non-transfusion-dependent thalassaemia (NTDT), who are being administered hydroxycarbamide or other agents to raise the Hb F percentage, or who have had gene therapy. HPLC or CE is satisfactory but it should be noted that some programmes on some HPLC instruments underestimate Hb F levels (see below).
- In the investigation of other unexplained haematological disorders or laboratory findings (see Table 4).

**Recommendation**

- Patients newly arrived in the United Kingdom with a history of sickle cell disease, thalassaemia or unexplained

**Table 4** Clinicopathological features indicating the need for haemoglobinopathy investigations.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Suspected globin chain disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydropic foetus</td>
<td>Haemoglobin Bart's hydrops fetalis</td>
</tr>
<tr>
<td>Neonate or infant with anaemia and either Hb F only or unexpectedly low percentage of Hb A</td>
<td>β thalassaemia major/NTDT</td>
</tr>
<tr>
<td>Unexplained anaemia and splenomegaly</td>
<td>β thalassaemia major/NTDT or intermedia/NTDT, haemoglobin H disease, unstable haemoglobin</td>
</tr>
<tr>
<td>Suspected thalassaemia or unexplained microcytosis</td>
<td>Thalassaemia or thalassaemic haemoglobinopathy including haemoglobin H disease, haemoglobin E and haemoglobin Lepore</td>
</tr>
<tr>
<td>Clinical and haematological features suggestive of sickle cell disease</td>
<td>Sickle cell disease (sickle cell anaemia and compound heterozygous states)</td>
</tr>
<tr>
<td>Unexplained haemolysis</td>
<td>Haemoglobin H disease, unstable haemoglobin</td>
</tr>
<tr>
<td>Unexplained target cells</td>
<td>Thalassaemia, variant haemoglobin</td>
</tr>
<tr>
<td>Unexplained irregularly contracted cells</td>
<td>Variant haemoglobin, particularly haemoglobin C, haemoglobin E or an unstable haemoglobin</td>
</tr>
<tr>
<td>Unexplained polycythaemia</td>
<td>High-affinity haemoglobin</td>
</tr>
<tr>
<td>Unexplained cyanosis with normal oxygen saturation</td>
<td>Methaemoglobins</td>
</tr>
</tbody>
</table>

Abbreviations: NTDT, non-transfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia.
anaemia should be investigated appropriately. (1A)

- Patients receiving blood transfusions or hydroxy-carbamide for the management of sickle cell disease should be appropriately monitored. (1A)

- Patients with β thalassaemia intermedia/NTDT being administered hydroxy-carbamide or having received gene therapy should have haemoglobin F monitored. (1A)

- Clinical and laboratory staff should be alert to the need for haemoglobinopathy/thalassaemia investigations when clinical or laboratory features indicate that a significant abnormality may be present. (1B)

- Laboratories should be aware of the alterations induced by voxelotor therapy in patients with sickle cell anaemia and, when necessary, should report the total percentage of a normal or variant haemoglobin. (1B)

Laboratory methods

The availability of fully automated systems and reagents for techniques, such as HPLC and CE, has led to their introduction in a large proportion of laboratories, replacing cellulose acetate electrophoresis (CAE) as a first-line screening method. The use of mass spectrometry is becoming more widespread for variant identification and is now used for newborn screening.

The choice of methodology and equipment will be based on volume of workload, sample material (liquid blood or dried blood spots), ease of handling, reproducibility, local availability and expertise and cost. The principles and methodology of the techniques used for haemoglobin analysis are outside the scope of this guideline but are available in standard textbooks.

High-performance liquid chromatography

High-performance liquid chromatography can be used for the quantification of haemoglobins A, S, A2 and F and for the detection, provisional identification and quantification of many variant haemoglobins. HPLC usually provides accurate quantification of Hb A2 and is, therefore, suitable for the diagnosis of β thalassaemia trait. Automated HPLC systems are often used as the initial diagnostic method in laboratories with a high workload.

High-performance liquid chromatography usually separates haemoglobins A, A2, F, S, C, D-Punjab and G-Philadelphia from each other. However, both Hb E and Hb Lepore often co-elute with A2 (as other haemoglobins co-elute with A, S and F) but can be recognised by alternative techniques. HPLC has the potential disadvantage that it also separates glycosylated and other derivative forms of haemoglobin from unmodified haemoglobin, which can make interpretation more difficult. For example, derivatives of haemoglobin S may co-elute with haemoglobin A2, rendering its quantification inaccurate. However, it should be noted that with this technique the detection of previously unsuspected diabetes mellitus is not infrequent. Careful examination of every chromatogram is essential with the report drawing attention to the presence of an increased percentage of glycosylated haemoglobin. The percentage of Hb A should be reported but it must be noted that glycosylated Hb S appears in the A2 window (up to 2%-3% is not unusual) and this should not be misidentified as A3. The A2 window is that of unmodified Hb A. Reporting of this low percentage of haemoglobin in the A2 window in a patient with sickle cell anaemia or sickle cell/beta thalassaemia is not advised since it is likely to cause confusion to clinical staff. It should also be noted that many HPLC instruments do not quantitate Hb H or identify adducts of Hb F; not only is Hb F underestimated but, in the case of haemoglobin H, the total of normal and variant Hbs will be less than 100%. It should be noted that in other cases also the sum of the variant and normal haemoglobins identified may be more or less than 100%. As with every method of haemoglobin analysis, controls should be run with every batch. Identification of variants is only provisional, and complementary second-line methods should be used to increase the confidence in the result. Confirmation can be obtained using DNA or protein sequencing techniques.

If HPLC is used as the screening technique, it is essential to check and maintain the positions of the windows, which are used as the first stage identification of any variants found. When required for a specific programme or instrument, this should be done following the manufacturer’s instructions. This is just as important as the calibration of the Hb A2 and Hb F levels and should be checked daily or as recommended by the manufacturer. Appropriate controls should always be included. If HPLC is used as the screening technique, CE or electrophoresis at alkaline and acid pH is suitable as a confirmatory technique or, if sickle cell trait is suspected, a sickle solubility test.

Capillary electrophoresis

Capillary electrophoresis can be used for the quantification of haemoglobins A, S, A2 and F and for the detection, provisional identification and quantification of many variant haemoglobins. CE usually provides accurate quantification of Hb A2 and is, therefore, suitable for the diagnosis of β thalassaemia trait. It separates Hb E from Hb A2 and permits the accurate measurement of Hb A2 in the presence of Hb E (but not Hb C). Automated CE systems are being used increasingly as the initial diagnostic method in laboratories with a high workload, facilitating a larger workload than is possible with HPLC. Optical density levels greater than 0.07 and the presence of either Hb A and Hb A2 or Hb F and Hb A2 is required to determine the migration position and thus permit ‘zoning’ and a provisional identification of haemoglobins present in the sample. CE does not separate glycosylated fractions, which may be considered an advantage. It should be noted that because HPLC separates post-translationally
modified haemoglobins and CE does not, the percentages of normal and variant haemoglobins are likely to differ between the two methods.

Capillary electrophoresis usually separates haemoglobins A, A₂, E, F, S, C, D-Punjab and G-Philadelphia from each other (but often with the overlap of C and A₃). Careful examination of every chromatogram is essential. As with every method of haemoglobin analysis, controls should be run with every batch. Identification of variants is only provisional, and complementary second-line methods should be used for confirmation.

If CE is used as the screening technique, HPLC is suitable as a confirmatory technique or, if sickle cell trait is suspected, a sickle solubility test.

Isoelectric focusing

Isoelectric focusing is satisfactory for the analysis of whole blood samples, haemolysates or dried blood spots. IEF gives good separation of Hb F from Hb A and clinically significant variant haemoglobins (S, C, D-Punjab, E and O-Arab). IEF can be semiautomated, rendering the technique suitable for screening large numbers of samples. However, this technique has not been validated for Hb A₂ quantification.

Although IEF has better resolution and the advantage that it separates more variants than CAE, it also has the disadvantage that it separates haemoglobin into its post-translational derivatives, for example Hb F separates into F₁ (acetylated) and F₁₁, Hb A can separate into A₀, A₁, A₁₁, A(αmet), A(βmet) and A(αβmet)—and similarly for other haemoglobins. This makes interpretation more difficult. Identification of variants is still only provisional, and second-line methods should be used for further analysis.

Cellulose acetate electrophoresis

Haemoglobin electrophoresis at pH 8.4–8.6 using a cellulose acetate membrane is simple, reliable and rapid but its use is in steady decline in high-income countries because of the frequent need to deal speedily with a large workload. It remains a valid method in resource-constrained settings and as a secondary technique following HPLC. It enables the provisional identification of haemoglobins A, F, S/G/D, C/E/O-Arab, H and a number of less common variant haemoglobins. Differentiation between haemoglobins migrating to a similar position can be obtained by using electrophoresis on acid (agarose) gels, HPLC or IEF. The provisional identification of any variant haemoglobin should be supported by at least one further unrelated method. Application of an alternative technique will exclude the possibility that a single band in either the S or C position represents a compound heterozygous state such as S/D or S/G and C/E or C/O-Arab respectively. If a patient has microcytosis the possibility that a single band represents compound heterozygosity for a variant haemoglobin and β thalassaemia must also be considered. Variant haemoglobins, such as Hb S can be quantified by scanning densitometry after electrophoresis/staining; however, quantification of haemoglobin A₂ by this method is not recommended as the precision is not good enough for the diagnosis of β thalassaemia trait.

Sickle solubility test

The kits for sickle cell solubility tests that are predominantly used in the United Kingdom will detect haemoglobin S down to a concentration of 20% (and sometimes below; in some cases as low as 8%). The method of Bain et al., although less sensitive than some commercial kits, can detect Hb S down to a concentration of 20%. The methods are, therefore, capable of detecting all cases of sickle cell trait beyond the period of infancy, even when there is co-existing α thalassaemia trait (but possibly not when there is co-existing Hb H disease). False positives have been described in patients with high plasma protein levels and in anaemic patients when double the volume of blood is used in the test. The latter problem can be avoided, however, by using a more concentrated sample of blood or by washing the red cells.

All positive and equivocal sickle solubility tests should be confirmed by HPLC, CE or an alternative technique both for confirmation of the presence of Hb S and to distinguish sickle cell trait from sickle cell anaemia and from compound heterozygous states. In an emergency, for example preanaesthesia, this distinction can be made with reasonable accuracy with a sickle solubility test combined with a blood film and a blood count. It is also recommended that all negative sickle solubility tests be confirmed by HPLC, CE or an alternative technique. Conversely, sickle solubility testing should be employed whenever an unknown haemoglobin is encountered.

In general, a sickle solubility test is not generally indicated in an infant before the age of 6 months because a negative result may be misleading. However, a sickle solubility test can sensibly be performed in an emergency, prior to anaesthesia, as if it is negative it is unlikely that anaesthesia will cause any clinical problems because the Hb S percentage will be too low. The wording of the report on such a test must state that a negative test does not exclude the presence of a low percentage of haemoglobin S and that further testing is necessary and will follow.

Other methods for detection of Hb S

Novel techniques using various devices are now available, which employ various methods to detect Hb S, Hb A and in some cases Hb C. These are based on density separation of cells, on the ability of cells to move through a chromatography membrane after mixing of the specimen with a sickle solubility reagent or on immunological techniques. These are primarily aimed as a point-of-care testing and are not designated as suitable for a first-line screening test for antenatal or newborn screening in England and we do
not recommend their use for this purpose in the United Kingdom. Some laboratories have chosen to replace the solubility test with these devices.

Investigation of patients taking voxelotor

The complexity of haemoglobinopathy investigations in patients with SCD being administered voxelotor should be noted. This drug binds to the alpha globin chain and thus alters the structure of haemoglobins A, A_2, F and S. This can lead to double peaks or bands, sometimes overlapping and sometimes distinct, on HPLC, CE and IEF (Figure 2). It is essential that the laboratory is aware that the patient is taking this drug in order for an informed and accurate report to be issued. Use of both HPLC and CE can facilitate interpretation. In patients who have been transfused it may be necessary to know the percentage of total haemoglobin A and total haemoglobin S. When there are two distinct peaks or bands, consideration should, therefore, be given to reporting in the format ‘haemoglobin A (or A_2) x% altered haemoglobin A y%’; when peaks are overlapping and are integrated the wording ‘haemoglobin A plus altered haemoglobin A x%’ can be used. Similar wording can be used for the percentages of haemoglobins S, F and A_2.

Detection of haemoglobin H bodies (Hb H bodies)

Haemoglobin H bodies are intracellular precipitates of haemoglobin with β_4 tetramers seen in red cells following supravital staining with brilliant cresyl blue or new methylene blue. They are seen in conditions where there is an excess of β globin chains, which nearly always results from a deficiency of a globin. They can be detected in carriers of α thalassaemia and in Hb H disease as well as in α thalassaemia and mental retardation (ATRX syndrome) and acquired Hb H disease.

Detection of Hb H bodies has been used to try and distinguish between homozygous α^+ (−/−α/α) and heterozygous α^- (−/αα). These two conditions are haematologically indistinguishable, although only the latter, in the homozygous state, can lead to a foetus with Hb Bart’s hydrops fetalis. Although Hb H bodies are much easier to find in α^0 heterozygosity than in α^- heterozygosity, this is an unreliable test, which is very time-consuming and has been superseded by DNA analysis. For this reason, many laboratories no longer offer this manual technique. However, detection of Hb H bodies remains useful to confirm the diagnosis of Hb H disease, in which golf-ball-like inclusions are seen in about 5% of red cells following supravital staining. It is also useful to look for Hb H bodies in cases of suspected ATRX syndrome or acquired Hb H disease. Both of these are typically suspected when there is otherwise unexplained microcytosis or hypochromia in the presence of characteristic clinical features, although confirming the diagnosis is difficult. Detection of Hb H bodies in these circumstances can be diagnostic. The number of Hb H bodies varies from very occasional in some cases of ATRX to more than 5% in acquired Hb H disease.

Hb A_2 measurement

High-performance liquid chromatography or CE can be used to quantify Hb A_2. Electrophoresis with elution is not suitable for routine diagnostic use. Microcolumn chromatography is acceptable but is unnecessary when HPLC or CE is the primary technique. IEF and scanning densitometry are not acceptable. No confirmatory test is necessary if the Hb A_2 is raised (but <8%) and the red cell indices are typical of β thalassaemia trait. Hb Lepore and Hb E may appear in the A_2 window on HPLC and should be considered if the level is apparently over ~6%–8%. Occasional other haemoglobin variants appear in the A_2 window so any value of ~6%–8% or greater should be assessed further. In cases of a split A_2, a second-line test will be required to identify an alpha chain variant. This may require genetic testing. However, when a delta variant is suspected, genetic testing is not necessary and the total Hb A_2 is simply calculated by adding the two A_2 peaks.

Quantification of Hb F

Quantification of Hb F is indicated if raised Hb F is detected beyond infancy, for example in SCD, thalassaemia major/TDT or intermedia/NDTDT, suspected HPFH, suspected δβ thalassaemia and suspected neoplastic or bone marrow failure syndromes, such as juvenile myelomonocytic leukaemia and Diamond–Blackfan anaemia. A Kleihauer test may assist in the differentiation of these conditions although DNA testing has superseded its use in the United Kingdom. However, a Kleihauer test can still be used to confirm that Hb in the F window on HPLC or CE is actually Hb F. Flow cytometry can also be used to quantify the number of F cells, as an alternative to the Kleihauer test. The 2-min alkali denaturation test is suitable for the quantification of Hb F levels <15%, but significantly underestimates higher levels and is now little used. Quantification by HPLC has the disadvantage of separating and thus excluding the added (post-translational) derivatives of Hb F, which can lead to a significant underestimate at high levels. Additionally, there can be interference from Hb A_2 and from some variant haemoglobins that co-elute with Hb F by HPLC. With CE, adducts of Hb F are included in the estimate of Hb F.

Selection of laboratory methods

Screening for variant haemoglobins

The analytical procedures employed must be capable of detecting all the common clinically significant haemoglobin variants, that is S, C, D-Punjab, E, O-Arab and Lepore and must be suitable for screening for thalassaemia. The following techniques are suitable for use in antenatal screening for haemoglobin variants in the United Kingdom:
FILURE 2 High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in a patient receiving voxelotor who has been transfused. (A) HPLC showing two distinct haemoglobin A peaks (‘unknown’ and A0) and two overlapping haemoglobin S peaks (‘unknown and S window’). (B) CE showing a haemoglobin S peak of abnormal shape and two distinct haemoglobin A2 peaks (E zone and A2). With thanks to Yvonne Daniel and Synnovis, Guy’s and St Thomas’ Hospital.
• High performance liquid chromatography;
• Capillary electrophoresis.

Abnormal results should be confirmed by a complementary technique that is appropriate for the likely variant. Other techniques that can be used for confirmation, besides those listed above, are IEF, cellulose acetate or acid agarose electrophoresis, or DNA analysis, although these are not suitable as screening techniques.

Sickle solubility testing or other methods specific for Hb S can be used as confirmation of an initial screen that suggests the presence of sickle haemoglobin (see above).

Screening for thalassaemia

Methods used are red cell indices in conjunction with the measurement of Hb A₂ and Hb F percentages. Routine measurement of indices includes measurements of MCH and mean cell volume (MCV); it is recommended that MCH is used to screen for thalassaemia as this parameter is more stable than MCV. These measurements are reported for all commonly used automated instruments. Hb A₂ is quantified by HPLC or CE. While the Hb F may be raised, it is the Hb A₂ percentage that is most significant for the diagnosis of β thalassaemia trait. Hb F percentage is particularly important in the diagnosis of δβ thalassaemia.

Selecting an HPLC or CE system

When choosing an HPLC or CE system, some general considerations need to be borne in mind.

A national recommended value of Hb A₂ of 3.5% or above has been set as the action point in the diagnosis of carriers of β thalassaemia in the antenatal screening programme. Two values for Hb F have been set for the investigation of a raised foetal haemoglobin in pregnancy (5% and 10% depending on the MCH). The chosen system must, therefore, be able to measure Hb A₂ and Hb F with accuracy and precision at these action values and detect the haemoglobin variants as specified by the screening programme. Peaks should be clearly separated for accurate quantification. Laboratories should understand how integration takes place and be aware that HPLC peaks measured on sloping baselines or on shoulders of adjacent peaks are likely to be less reliable.

High-performance liquid chromatography and CE systems must be able to detect Hb A₂ variant peaks, due to α or δ chain variants. For the purposes of screening these should be added into the total Hb A₂ percentage when possible (for α chain variants this may not be possible). In a patient with an MCH below the cut-off point (≤27 pg), investigation of the baby’s biological father will be required if the total Hb A₂ is above 3.5%.

If using equipment or an elution programme for more than one analyte, for example Hb A₂ and Hb A₁₅, laboratories should ensure that the quantification of Hb A₂ and Hb F is not compromised. This may require a different column/buffer system.

Problems with the measurement and interpretation of Hb A₂

Besides the difficulties associated with the separation and quantification of small peaks or bands in any chromatographic or electrophoretic system, some other factors need to be considered when interpreting Hb A₂ results.

With many HPLC systems, Hb A₂ is overestimated in the presence of Hb S. However, increased Hb A₂ levels are not a reliable way of distinguishing between Hb SS and Hb S/β thalassemia. This is not a problem because in sickle cell trait the percentage of Hb A is greater than Hb S and the reverse is true in Hb S/β thalassemia.

Hb A₂ values >4.0% with normal indices may indicate β thalassemia trait with or without co-existing α thalassemia, or with co-existing conditions such as vitamin B₁₂ or folate deficiency. In this case:

• Re-analyse FBC and Hb A₁₅ on a repeat sample.
• Consider vitamin B₁₂/folate deficiency, drugs, liver disease/alcohol or HIV infection.

In the case of preconceptual or antenatal testing, consider the results in the baby’s biological father and, for antenatal screening, arrange DNA analysis without delay when appropriate. The α thalassemia risk needs to be considered in the light of the family origins. The major risk is for β thalassemia, but the risk of Hb Bart’s hydrops fetalis should not be overlooked.

Hb A₂ values ≤4.0% with normal red cell indices and a normal Hb F level can usually be regarded as normal, although some mild β thalassaemia alleles (mainly in subjects of Mediterranean origin) are associated with an A₂ of 3.5%–4.0%. In the context of antenatal screening, testing of the baby’s biological father should be requested in these cases.

Interpretation of results in the presence of iron deficiency.

Severe iron deficiency anaemia (haemoglobin concentration < 80 g/L) can reduce the Hb A₂ level slightly (by up to 0.5%). Outside of pregnancy, anaemia should be treated and the haemoglobin analysis repeated when the patient is iron replete. In pregnant women, there is no justification for delaying the investigation for haemoglobinopathies whilst treating iron deficiency presumptively, as this will delay the process of identifying at-risk carrier couples, who could be offered a prompt prenatal diagnosis. It may be appropriate to simultaneously investigate pregnant women for iron deficiency, for example using serum ferritin, but this is not specifically part of haemoglobinopathy screening.

Newborn screening. The NHS Sickle Cell and Thalassaemia Screening Programme advises that all neonates be offered screening for SCD.

The dried blood spot card used for sickle screening is the same as used for the other newborn screening programmes and samples are taken at 5 days. When clinically
indicated, such as in the situation of a high-risk pregnancy or when a baby is about to be transfused, testing in advance of screening can be carried out on the baby using a liquid sample direct from the baby. This must be done in a laboratory experienced in the interpretation of newborn samples. Three methods of analysis are currently recommended for first-line newborn screening for sickle cell disorders from dried blood spot samples: tandem mass spectrometry (MSMS), HPLC or CE. Screen-positive samples should be retested using an alternative procedure, which may include IEF, although this is not recommended following MSMS.

When using the percentages of the haemoglobin fractions to interpret the results, the possibility of the presence of transfused blood should also be considered.

The analytical procedures employed must be capable of detecting all the common clinically significant haemoglobin variants, that is S, C, D-Punjab, E and O-Arab, in addition to Hb F and Hb A. Neonatal samples are typically composed of mostly Hb F (approximately 75%) with approximately 25% Hb A and small quantities of acetylated Hb F and sometimes of Hb Bart’s. The procedures used must, therefore, be sensitive, reliable and reproducible in terms of detecting small quantities of Hb A and the abnormal haemoglobin fractions listed, in the presence of large amounts of Hb F.

It is also important to realise that occasionally the presumptive identification of a haemoglobin variant using screening methods is incorrect because some variants give exactly the same results using current screening techniques. The sensitivity and specificity are approximately 99% for the methods used. Unequivocal identification of haemoglobin variants can only be achieved by either protein analysis (e.g. mass spectrometry) or DNA analysis.

**Recommendations**

- Abnormal laboratory screening results should be confirmed by a complementary technique that is appropriate for the likely abnormality. (1A)
- Laboratories performing antenatal screening should utilise methods capable of detecting significant variants and be capable of measuring Hb A2 and Hb F at the action values required by the national antenatal screening programme. (1A)
- Quantification of Hb A2 by CAE plus scanning densitometry is not recommended and CAE plus elution is not generally appropriate. (1A)
- A sickle cell solubility test is not generally indicated in infants below the age of 6 months and is not recommended as a primary screening tool except in an emergency situation. All sickle solubility tests should be confirmed by HPLC, CE or an alternative method. (1B)
- Assessment of iron status may be useful in the interpretation of laboratory tests but should not delay testing of the baby’s biological father in the antenatal screening programme. (1A)
- Examination for Hb H bodies cannot reliably distinguish between α thalassaemia traits and should not be used for screening. (1B)
- Laboratories should be aware of the conditions that may not be detected when using the Antenatal and Newborn Screening Programmes’ algorithms and also of the effect of blood transfusion on the interpretation of results. (1A)

**Quality assurance and laboratory standards**

*External quality assessment*

External Quality Assessment (EQA) provides a long-term, retrospective assessment of laboratory performance, allowing laboratories to demonstrate consensus with their peers and providing information on intermethod comparability. Participation in EQA is required for laboratory accreditation and is an essential part of clinical governance.

Public Health England policy and ISO 15189 require both antenatal and newborn screening laboratories to participate in an accredited EQA scheme and to demonstrate satisfactory performance.

Laboratory standards for antenatal and newborn screening have been defined by the NHS Sickle Cell and Thalassaemia Screening Programme and are available in their laboratory handbook.3

**Indications for DNA analysis**

The policy guidance developed by the National Screening Programme means that for screening DNA analysis is needed only in a limited number of cases and not usually in those where α thalassaemia is suspected. The majority of couples at risk of having a child affected with β thalassaemia or SCD should be identified initially by routine laboratory techniques through the antenatal screening programme.

DNA analysis can be useful to confirm the precise diagnosis of SCD when the results of haemoglobin analysis are not available on both parents, or there is uncertainty about the diagnosis. Similarly, DNA analysis is increasingly used to diagnose rarer haemoglobinopathies, including unstable haemoglobins, methaemoglobins and altered-affinity haemoglobins. This can involve the direct sequencing of alpha or beta globin genes, the use of next-generation sequencing DNA panels and specific techniques to identify large deletions.

The diagnosis of α thalassaemia is more complicated than that of β thalassaemia because rapid methods for its detection are not yet widely available and because DNA analysis is the only accurate way to distinguish between α and α+ thalassaemia. Furthermore, non-deletional forms of α thalassaemia are more common than was previously thought. However, it is not practical or necessary to seek to confirm all potential cases of α thalassaemia by DNA
analysis because the α form is too common and not usually clinically important.

Table 5 summarises the main genetic risk combinations that require antenatal screening actions, based on the antenatal screening recommendations and indicates which cases require referral of samples for further studies by DNA analysis. For other haemoglobinopathy combinations, results should be assessed by a consultant expert in the field.

### Standardised reporting

Testing for haemoglobinopathies is a complex area because of the large number of haemoglobin variants and thalassaemic conditions. The NHS Sickle Cell and Thalassaemia Screening Programme has produced a set of report formats for both newborn and antenatal screening that should allow all normal and 95% of abnormal results to be reported in a standardised manner. However, because of the diversity of haemoglobin variants and thalassaemia syndromes, there will always be some situations that require further tests on different specimens or family studies before a conclusive diagnosis can be achieved.

**General notes on reporting screening results:**

- The sample date must be given (this can be extremely important if a person has had a recent blood transfusion).
- If a blood transfusion has been received within 4 months, misleading data and conclusions may result. This includes in utero transfusions. Consideration should be given to mentioning this possibility when haemoglobinopathy results are reported.
- Analytical fact should be separated from interpretative opinion. The factual results should be given first and

<table>
<thead>
<tr>
<th>Maternal carrier state</th>
<th>Biological father’s carrier state</th>
<th>Further studies by DNA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abnormalities detected</td>
<td>Testing of baby's biological father not required</td>
<td>None required</td>
</tr>
<tr>
<td>Any variant Hb</td>
<td>No abnormality detected</td>
<td>None required</td>
</tr>
<tr>
<td>Hb S</td>
<td>Hb S or Hb C</td>
<td>None required until PND</td>
</tr>
<tr>
<td>Hb S</td>
<td>Hb O-Arab, D-Punjab, E, Lepore, β or δβ thalassaemia</td>
<td>If PND is being considered, send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb S</td>
<td>Hb S</td>
<td>Send bloods for mutation confirmation of HPFH; PND is not usually indicated when this has been confirmed</td>
</tr>
<tr>
<td>Hb S plus probable α thalassaemia</td>
<td>Assess risk as per Hb S alone, unless family origins indicate a high risk of α-thalassaemia</td>
<td>Assess risk as per Hb S alone, unless family origins indicate a high risk of α-thalassaemia</td>
</tr>
<tr>
<td>Hb C</td>
<td>Hb S</td>
<td>None required until PND</td>
</tr>
<tr>
<td>Hb D-Punjab</td>
<td>Hb S</td>
<td>If PND is being considered send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb O-Arab</td>
<td>Hb S</td>
<td>If PND is being considered send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb Lepore</td>
<td>Hb S, E, Lepore, β thalassaemia or δβ thalassaemia</td>
<td>If PND is being considered, send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb E</td>
<td>β thalassaemia, Hb Lepore, δβ thalassaemia or Hb S</td>
<td>If PND is being considered, send bloods for mutation confirmation; if paternal MCH is &lt;25 pg, send blood for mutation analysis if there is high risk for α-thalassaemia</td>
</tr>
<tr>
<td>β or δβ thalassaemia</td>
<td>Hb S, E, Lepore, β thalassaemia or δβ thalassaemia</td>
<td>Send bloods for mutation confirmation of β thalassaemia and Hb Lepore; if paternal MCH is &lt;25 pg, send blood for mutation analysis if there is high risk for α-thalassaemia</td>
</tr>
<tr>
<td>Suspected HPFH</td>
<td>Hb S, E, Lepore, β or δβ thalassaemia</td>
<td>Send bloods for HPFH confirmation; if confirmed, PND is not usually indicated</td>
</tr>
<tr>
<td>Suspected α thalassaemia (MCH &lt;27 pg but ≥25 pg)</td>
<td>Testing of baby’s biological father not required</td>
<td>None required</td>
</tr>
<tr>
<td>Suspected α thalassaemia (MCH &lt;25 pg)</td>
<td>Testing of baby’s biological father not required</td>
<td>None required</td>
</tr>
</tbody>
</table>

1. Low risk of α thalassaemia in either biological parent based on family origin
2. Risk of α thalassaemia in both biological parents based on family origin or family origin unknown

- If paternal MCH <25 pg, send maternal and paternal bloods for mutation confirmation regardless of any other abnormality detected.
Abbreviations: HPFH, hereditary persistence of foetal haemoglobin; MCH, mean cell haemoglobin; PND, prenatal diagnosis.
should be followed by a clear conclusion, which may include recommendations. If there is likely to be a delay in producing a final result, an interim result should be issued, which may be sufficient for the clinician to move forwards with that patient’s clinical care.

- Consideration should also be given to adding the comment on all newborn reports where there is nothing abnormal detected that the presence of Hb F is normal in newborns.
- If information from the blood count is used in coming to a conclusion about the significance of the analytical data (as in probable α thalassaemia) then those aspects of the blood count used (such as haemoglobin concentration, red cell count, MCH, MCV) must be included in the haemoglobinopathy report.
- Similarly, if information on ethnicity/family origin is used, it should be stated in the report.
- Results of the sickle solubility test, in the absence of results from an unrelated confirmatory method, should be reported as an ‘interim’ report when there is an urgent clinical need for the information. The report should state that the result is provisional and that confirmation will follow. The final report with information from the blood film, HPLC and/or CE and any other appropriate tests should follow as soon as possible.
- As it improves clarity, the conclusion should always be given both in full text and in standard abbreviation form in parentheses. For example: sickle cell carrier (AS) or sickle cell anaemia (SS). The convention recommended is for the Hb initials to be reported in the order of greatest to least percentage.
- For antenatal samples, if no further action is required it may be helpful to say so or to state that testing of the baby’s biological father is not indicated.

Limitations of haemoglobinopathy diagnosis

The clinical diagnosis is based on combining the clinical picture with all the laboratory results with an understanding of the limitations of haematological, protein and DNA analyses.

Sensitivity/specificity

None of the current screening techniques can identify all abnormalities but the combined sensitivity/specificity of the HPLC, CE, mass spectrometry and IEF techniques for haemoglobins present at the time of screening is very high. The position of elution or zoning is not unique for a particular haemoglobin variant; however, for the purpose of screening most can be characterised sufficiently using the second complementary technique. Some cases will require DNA analysis for more precise identification. For example, Hb D will only be of significance if found to be Hb D-Punjab, but for the purposes of the screening programmes, further testing is only required if Hb D occurs in combination with Hb S in an individual or in a pregnant woman and the baby’s biological father. Nevertheless, since Hb D-Punjab may be relevant to other family members, offering definitive identification should be considered.

Recent blood transfusion

Interpretation of haemoglobin analysis is potentially misleading after a recent blood transfusion and necessitates repeat testing after 4 months. If the result is needed more urgently, DNA testing is recommended. For newborn screening, DNA testing for the sickle gene is now performed for transfused neonates to avoid the need for a repeat specimen at 4 months post-transfusion although a pretransfusion specimen is preferred. Laboratory staff undertaking testing should utilise information from their laboratory computer records where a possible recent blood transfusion may have occurred.

Conditions not detected by the newborn screening programme

The following conditions will only be detected once the mature haemoglobin pattern has developed:

- β thalassaemia carriers, as Hb A2 levels in the newborns are below the levels of reliable detection/quantification.
- Hb Lepore, since as for β thalassaemia carriers the gene expression is too low for reliable detection of the variant in newborns.
- Hereditary persistence of foetal haemoglobin, as newborns all have large amounts of Hb F with considerable variability in the levels.
- Some cases of thalassaemia major/TDT and intermedia/NTDT, in which significant amounts of Hb A are present at birth.

Conditions not differentiated by the newborn screening programme

The following conditions cannot be clearly differentiated until the mature haemoglobin pattern develops and, in some instances, until red cell indices or family studies are available:

- Homozygous Hb S versus Hb S/β+ thalassaemia, Hb S/β thalassaemia, Hb S/γβ thalassaemia, Hb S/εβ thalassaemia, Hb S/Hb Lepore and some cases of Hb S/β thalassaemia, as all can produce an FS pattern in the neonate; further diagnostic testing is required.
- Hb E/β+ thalassaemia versus Hb E/E, as only Hb F and Hb E will be present.
- Other possible heterozygous, compound heterozygous or homozygous states including those of less or no clinical significance.

Premature babies

Haemoglobin A is usually detectable at 30 weeks gestation and is sometimes detectable at 24 weeks. Very premature babies may not have any Hb A present and, therefore, will need repeat testing to ensure Hb A does develop and that
they do not have β thalassaemia major/TDT. Hb S and other β chain variants are generally present at lower levels than Hb A so may not be apparent in a very premature baby. Prematurity may also add to the difficulty in differentiating a sickle cell carrier from a baby with Hb S/β-thalassaemia when the Hb S level is greater than the Hb A level. Family studies and DNA testing may be helpful to make a diagnosis.

**Conditions not detected by the antenatal screening programme**

The following conditions will not be detected when following the algorithms for either a low or a high-prevalence area:

- silent/near silent β thalassaemia carriers
- α-thalassaemia carriers in non-high-risk groups
- dominant haemoglobinopathies in the biological father of a baby whose mother has a negative screen
- some uncommon but clinically significant haemoglobinopathies, for example unstable or altered-affinity haemoglobins
- coinheritance of β thalassaemia and triplicated α in a neonate

The following conditions are additional risks when using the algorithm for low-prevalence areas:

- haemoglobin variants in northern European families
- thalassaemia carriers obscured by vitamin B12/folate deficiency, liver disease or other causes of a rise in the MCV/MCH
- combined β thalassaemia carrier and α thalassaemia carrier in low risk groups, because a normal MCH would mean no HPLC or CE analysis is performed

The antenatal programme is not designed to detect couples at risk of a child with Hb H disease. Reporting of newborns with high Hb Bart’s is no longer recommended by the programme.

**Accurate information of family origin**

The antenatal programme is reliant on the quality of information supplied from the family origin questionnaire. Incorrect information in the high-prevalence areas may result in incorrect interpretation and subsequent inappropriate action, particularly with respect to potential α-thalassaemia carriers. In low-prevalence areas, incorrect information could mean that women miss screening completely.

The quality of this information is dependent on midwife knowledge and training to ensure that women understand the importance of revealing this information and consider the ethnic/family origin, not country of birth and not just of themselves and their partners, but also of earlier generations when known. However, some patients and partners may be unaware of their ethnic origin and sometimes the use of a donor egg or donor sperm is not reported. Where the family origin is unknown or a woman declines to reveal it, patients must be treated as high-risk.

**Linkage of neonatal and antenatal screening programmes.**

The original commitment in the White Paper was to establish ‘a linked antenatal and newborn screening programme for haemoglobinopathies and SCD’. The current national policy requires that users should experience one service, not separate disjointed services, despite the fact that this must be delivered by separate regional newborn and locally provided antenatal programmes. A key objective for the linkage is to minimise the adverse effects of screening—anxiety, misunderstanding, inaccurate information, unnecessary investigation and irrelevant follow-up—and this can only be achieved by good communication between the two screening services.

**Method of linkage of neonatal and antenatal screening programmes**

All maternity units are required to have a local policy applying national guidance on the integration of antenatal and newborn screening programmes. To link an SCT antenatal screening carrier result and/or diagnostic result with the newborn blood spot taken on day 5, units must complete and send the at-risk pregnancy alert form to the newborn screening laboratory.

Local agreement is required between the antenatal screening coordinator and screening laboratories as to who should be notified in the newborn screening laboratory and by whom.

It is equally important that a method is established for communicating positive results back to the relevant antenatal services and laboratories, particularly in low-prevalence areas where specialist haemoglobinopathy nurses may not be available to ensure that results are reviewed.

**Recommendations**

- A local written policy for linkage of antenatal and neonatal screening services is required, including the use of the at-risk pregnancy alert form. (1A)
- There should be good communication between antenatal and neonatal screening services since this may help to reduce unnecessary anxiety, possible misunderstandings and missed diagnoses and allow detection and investigation of possible errors. (1A)

**Haemoglobinopathy cards and registry**

Haemoglobinopathy cards are available for affected, carrier and normal individuals following haemoglobinopathy screening. It is considered good practice to issue haemoglobinopathy cards to those individuals with a major haemoglobinopathy and also to carriers where a definitive diagnosis can be made.

The issue of ‘normal’ cards to individuals with no evidence of a significant haemoglobinopathy should be risk assessed by
each centre. The national screening programmes have been developed specifically to detect major clinically significant haemoglobinopathies; exclusion of these on such screening programmes does not necessarily equate to normal.2

In the absence of confirmatory DNA testing, patients should not be issued with haemoglobinopathy cards stating that they are carriers of α thalassaemia or probable carriers of α thalassaemia. Units may wish to consider issuing explanatory letters or leaflets for this group of patients. Patients diagnosed with SCD and clinically significant thalassaemia should also be entered in to the National Haemoglobinopathy Registry, which will normally be done by the relevant clinician.

Recommendations

1. Haemoglobinopathy cards should be issued to individuals with a major haemoglobinopathy. (1A)
2. Haemoglobinopathy cards should be issued to carriers where a definitive diagnosis can be made. (1A)
3. Haemoglobinopathy cards should not be issued to individuals with apparent α thalassaemia unless confirmed by DNA testing. (1A)

AUTHOR CONTRIBUTIONS

Barbara J. Bain, Yvonne Daniel, Joan Henthorn, Barbara de la Salle, Amanda Hogan, Noémi B. A. Roy, Ciaran Mooney, Lisa Langabeer and David C. Rees—all contributed equally to the data synthesis and writing of this guideline.

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CONFLICT OF INTEREST STATEMENT

All authors have made a full declaration of interest to the BSH and Task Force Chairs, which may be viewed on request. None of the authors have any relevant conflicts of interest to declare.

DISCLAIMER

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, the British Society of Haematology nor the publishers can accept any legal responsibility for the content of these guidelines.

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REFERENCES


APPENDIX A

A.1 | Search terms

Sources searched: Medline, Embassy, Cochrane Library, National Library for Health

Search strategy

Ovid Medline

1. (Sickle adj cell1).two.
2. (sickle adj trait1).tw.
3. (haemoglobin adj s).tw.
4. (haemoglobin adj s).tw.
5. (sickle adj disease).tw.
6. exp Anaemia, Sickle Cell/
7. (haemoglobinopath or haemoglobinopathy).tw.
8. (thalassaemia or thalassemia).tw.
9. exp Haemoglobinopathies/
10. exp Thalassemia/
11. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12. screen3.tw.
13. (test or tests or testing).tw.
14. detect3.tw.
15. opportunistic.tw.
16. targeted.tw.
17. universal.tw.
18. selective.tw.
19. mass screening/or genetic screening/or neonatal screening/
20. exp Genetic Counselling/
21. (counselling or counselling).tw.
22. prevalence/
23. exp Sensitivity/and Specificity/
24. nanotechnology.tw.
25. exp Nanotechnology/
26. exp Spectrum Analysis, Mass/
27. (tandem MS or tandem mass spectrometry).tw.
28. exp Follow-Up Studies/
29. stroke prevention.tw.
30. (genetic risk or clinical risk).tw.
31. isoelectric focusing.tw.
32. (HPLC or high-performance liquid chromatography).tw.
33. (family origin or question).tw.
34. exp Genetic Predisposition to Disease/
35. (bloodspot or blood spot).tw.
36. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35
37. (antenatal or pregnan).tw.
38. neonat2.tw.
39. preconcept.tw.
40. population.tw.
41. carrier.tw.
42. (homozygous or heterozygote1).tw.
43. Homozygote/or Heterozygote/
44. Prenatal Diagnosis/or Pregnancy/
45. exp Infant, Newborn/
46. 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45
47. 11 and 36 and 46
### APPENDIX B

#### B.1 Family origin questionnaire

**NHS Sickle Cell and Thalassaemia Screening Programme**

**Family Origin Questionnaire**

If using a pre-printed label please attach one to each copy

<table>
<thead>
<tr>
<th>Hospital number</th>
<th>NHS number</th>
<th>Estimated delivery date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname</td>
<td>Forename</td>
<td>Date of birth</td>
</tr>
<tr>
<td>Address 1</td>
<td>Address 2</td>
<td>Post code</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestation at time of sample (weeks and days)</th>
<th>Screening test declined</th>
<th>Report destination (such as community midwifery, GP, antenatal clinic, obstetrician)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Is pregnancy the result of IVF?** If yes, complete the form including **SECTION H**.

**What are your and your family's origins?**

Please tick all boxes in **ALL** sections that apply to the woman and the baby’s biological father.

**A. AFRICAN OR AFRICAN-CARIBBEAN (BLACK)**
- Caribbean Islands
- Africa (excluding North Africa)

**Biological father**

**B. SOUTHEAST ASIAN (ASIAN)**
- India or African-Indian
- Pakistan, Bangladesh, Sri Lanka

**Biological father**

**C. SOUTH EAST ASIAN (ASIAN)**
- China including Hong Kong, Taiwan
- Singapore, Thailand, Indonesia
- Malaysia, Vietnam, Philippines
- Cambodia, Laos, Myanmar
- Any other Asian family origins

**Biological father**

**D. OTHER NON-EUROPEAN (OTHER)**
- North Africa, South America
- Middle East, Saudi Arabia, Iran
- Any other non-European family origins

**Biological father**

**E. SOUTHERN AND OTHER EUROPEAN (WHITE)**
- Sardinia
- Greece, Turkey, Cyprus
- Italy, Portugal, Spain
- Albania, Czech Republic
- Portugal, Romania, Russia
- Any other Mediterranean country

**Biological father**

**F. UNITED KINGDOM (WHITE)** refer to the list on the back
- England, Scotland, Northern Ireland, Wales

**Biological father**

**G. NORTHERN EUROPEAN (WHITE)** refer to the list on the back
- Austria, Belgium, Switzerland, Scandinavia
- France, Germany, Netherlands
- Australia, North America, South Africa
- Any other European family origins

* Hb Variant Screening Requested by (F) and/or (G)

### Higher risk for alpha zero thalassaemia

**H. DON'T KNOW**

Adoption/unknown ancestry

Donor egg/sperm (if pregnancy results from donor egg, order test for mother and offer biological father test immediately)

Bone marrow transplant (if mother has had a bone marrow transplant, order test for mother and offer biological father test immediately)

**Biological father**

**I. DECLINED TO ANSWER**

All women need to be informed that routine analysis of blood may identify them as a thalassaemia carrier. In the **prevalence area OFFER** haemoglobin variant screening to all women if they or the baby's father have answers in any yellow box. In the **high prevalence area OFFER** haemoglobin variant screening to all women (independently of answers).

Signed: [signature]
Print name: [name]
Hospital: [hospital]
Date: [date]

(By health care professional completing the form)