Sickle Cell and Thalassaemia

Handbook for Laboratories

Incorporating:
• Policy Guidance
• Laboratory Standards
• Testing Algorithms
• Standardised Reporting Comments
• Referral Guidelines for DNA Analysis
• Procurement Details
• Risk Assessments

October 2012
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This is the third edition of the Laboratory Handbook and updates the second edition which was published in September 2009. It includes amendments and additions to the policies and guidance that have been made since then. In many cases this has simply meant ‘fine-tuning’ some aspects where further clarification was required. In some cases policy has been updated. Most of these updates have appeared on the screening programme website and have been published in Circulate. Information for laboratories is regularly updated in the laboratory update section of the screening website (sct.screening.nhs.uk/labupdates) and laboratories are encouraged to check this section frequently. It must be emphasised from the beginning that the policies described relate to a screening programme and not a diagnostic service. No screening programme can detect every haemoglobinopathy, i.e. have a diagnostic sensitivity of 100%, and therefore it is likely that a few cases will be missed. This must be borne in mind when counselling couples about the effectiveness of screening.

The programme centre is monitoring the sensitivity and specificity of the screening programme. Should any false positives or negatives come to your attention, irrespective of whether they constitute an incident, please notify us at: haemscreening@kcl.ac.uk

Since the first two editions, the laboratories have been requested to supply a great deal of audit data to assess the effectiveness of the screening programme. Key Performance Indicators (KPIs) have been defined to ensure that critical aspects of the screening pathway are being monitored. For many laboratories this has meant extra work when they are already hard pressed. Their diligence is appreciated and hopefully the burden will diminish as new protocols for gathering data become embedded in routine practice.

The British Committee for Standards in Haematology (BCSH) has published guidance about screening in a paper entitled ‘Significant haemoglobinopathies: guidelines for screening and diagnosis’, 2010, British Journal of Haematology, 149, 35–49. Every effort has been made to ensure that the BCSH guidelines and this edition of the Handbook are in complete accord.

We would like to thank all those who have contributed to the Handbook, in whatever capacity. Besides those listed as major contributors, there have been many others whose input has been of great help. Many thanks.

Although the Handbook has been written specifically for the screening programme in England, the policies are also being adopted in the other UK countries.

If you wish to reference or acknowledge this document, please use the following format; NHS Sickle Cell and Thalassaemia Screening Programme, Handbook for Laboratories, 3rd edition; October 2012.

As before, we would welcome your comments about the contents of this 3rd edition of the Handbook and also its value.

At the time of going to print, all information and contact details were correct. This edition is likely to be reviewed in April 2015.
Background to the haemoglobinopathies

The haemoglobinopathies are a heterogeneous group of more than 1,000 conditions, which are categorised into two main groups: the haemoglobin variants and the thalassaemias. The haemoglobin variants (also called the abnormal haemoglobins) arise from an alteration in the globin protein structure, whereas the thalassaemias arise from inadequate production of structurally normal globin protein. There are also thalassaemic haemoglobinopathies, e.g. haemoglobin E and haemoglobin Constant Spring, when a structurally abnormal haemoglobin is synthesised at a reduced rate. Sickle cell disease is caused by the inheritance of a variant haemoglobin and often results in severe life-threatening clinical symptoms. Those with β thalassaemia major require regular blood transfusions to maintain life.

There are to date over 1,000 different haemoglobin variants described and approximately 300 mutations giving rise to thalassaemia. Some of the abnormalities are easily detected by simple biochemical procedures, whereas others are biochemically silent. The frequency of different haemoglobinopathies varies in different ethnic groups and certain haemoglobinopathies are often associated with a family history. However, it is important to remember that no haemoglobinopathy is exclusive to any single ethnic group; thus all persons are theoretically at risk of carrying an abnormal gene. It is not unusual for people to inherit more than one haemoglobin abnormality and many populations are at risk for a range of affected genes. Many haemoglobinopathies are of no clinical significance whereas others are associated with severe morbidity and mortality, most notably sickle cell disease and β thalassaemia major; carriers are usually asymptomatic.

Sickle cell disease

Sickle haemoglobin (Hb S) is a haemoglobin variant in which the sixth amino acid on the beta globin chain, glutamic acid, is replaced by valine. Other much rarer haemoglobins have been reported which have this same glutamic acid to valine substitution but also an additional substitution elsewhere in the beta chain. All of these variants have a positive sickle solubility test, though their electrophoretic characteristics may be different, and all will cause sickle cell disease in the situations described below for Hb S. Sickle cell disease results from the inheritance of one of the following genotypes: homozygosity for Hb S (sickle cell anaemia); or compound heterozygosity for Hb S and an interacting gene such as Hb C (Hb SC disease), or β thalassaemia (Hb S/β thalassaemia). A list can be found in Table 1. The sickling disorders are associated with severe life-threatening vaso-occlusive crises, overwhelming sepsis, splenic sequestration, aplastic crises, stroke, priapism, pulmonary hypertension, proliferative retinopathy and chronic organ damage, such as avascular necrosis of the hips and shoulders.

There is substantial evidence that early administration of prophylactic penicillin markedly reduces the incidence of pneumococcal sepsis. There is also evidence that pneumococcal vaccines can increase immunity to pneumococcal infections in people with sickle cell disease. Penicillin V (or alternative) prophylaxis should be started from 1 month of age and conjugate pneumococcal vaccine, which is recommended for all children, should be given as per national immunisation schedules starting at 2 months of age. Audit standards require that 90% of infants should have been offered and prescribed prophylaxis by 3 months and 99% of infants by 6 months (NHS Sickle Cell & Thalassaemia Screening Programme, Sickle Cell Disease in Childhood: Standards and Guidelines for Clinical Care, 2010). Clinical monitoring to detect acute splenic sequestration reduces morbidity and mortality from homozygous sickle cell anaemia (Hb SS) in infancy and early childhood. Studies have demonstrated the benefits of early diagnosis by the widespread implementation of newborn screening programmes, parental education and comprehensive care for patients with sickle cell disease and β thalassaemia major.

General screening policy

The NHS plan for England published in April 2000 made the commitment to implement “effective and appropriate screening programmes for women and children
including a new national linked antenatal and neonatal screening programme for haemoglobinopathy and sickle cell disease”. The NHS Sickle Cell and Thalassaemia Screening Programme was set up as a consequence of this policy statement.

The screening programme is best seen as two linked parts: sickle cell and thalassaemia screening during pregnancy, and sickle cell screening in the newborn period. Sickle cell screening may also be carried out prior to general anaesthesia but is not part of this programme and is not considered further here.

**Screening helpline**

Laboratories may have questions about screening policy or interpretation of results that cannot be answered easily by reference to this handbook or textbooks. Oxford University Hospitals NHS Trust now provides a support service for screening laboratories via designated telephone help-lines and secure email. The service supports both antenatal and newborn screening enquiries. The new service started on 7 October 2010 and can be accessed by email, telephone or fax.

Designated telephone line: 01865 572 767
Secure email: lab.support@nhs.net
Secure FAX: 01865 572 775
Newborn Screening

The objective of the newborn screening programme is to detect infants at risk of sickle cell disease within the neonatal period, in order to allow early diagnosis and to improve outcomes through early treatment and care. It is essential that infants with these conditions are reliably diagnosed and that they are clearly reported as having a sickle cell disease (see Table 1) and that the necessary clinical follow up is arranged. The analytical methods used will also detect most cases of β thalassaemia major and related conditions. α and β thalassaemia carriers will not be detected.

Newborn sickle cell screening is offered to all babies born in England and is also being adopted in the other UK countries. The screening is offered at 5-8 days of age as part of the newborn dried blood spot screening programme (newbornbloodspot.screening.nhs.uk). The service is provided from centralised laboratories in the existing UK Newborn Screening Laboratories Network (UKNSLN), which already perform universal newborn screening for phenylketonuria (PKU), congenital hypothyroidism (CHT), cystic fibrosis (CF) and medium chain acyl CoA dehydrogenase deficiency (MCADD). (See Appendix 9 for a list of laboratories involved in newborn screening.)

The UK Newborn Bloodspot Screening Programme Centre has published generic standards for newborn screening, against which the screening services will be assessed (newbornbloodspot.screening.nhs.uk/standards).

Informed consent

An explanatory leaflet detailing the purpose, process and outcomes of newborn screening for sickle cell conditions must be provided to the parent(s) prior to screening. This should be as part of a leaflet explaining all aspects of the newborn bloodspot screening programme (Screening tests for you and your baby - newbornbloodspot.screening.nhs.uk/public). Screening should be explained by midwives during pregnancy and then again before taking the test. In cases where the infant’s parent(s) does/do not wish the child to be screened for sickle cell (or any of the other conditions), the decision to opt out of testing must be specifically documented on the bloodspot card. Since parents may wish to opt out of one of the tests in the screening process but not others, it is essential that the document clearly states which screening test the parent does not wish to be undertaken. Further guidance on informed consent in newborn screening has been developed by the UK Newborn Bloodspot Screening Programme (newbornbloodspot.screening.nhs.uk/consent).

Clinically significant haemoglobinopathies that must be detected

A large number of haemoglobin variants are detected using current screening methods. Those for which there is evidence that early intervention is likely to be beneficial, and which are therefore specified as part of the national screening programme, shown in Table 1 below:

Table 1: Sickle cell disease

<table>
<thead>
<tr>
<th>Sickle cell anaemia (Hb SS)</th>
<th>Hb SC disease</th>
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<tbody>
<tr>
<td>Hb S/β thalassaemia*</td>
<td>Hb S/DPunjab</td>
</tr>
<tr>
<td>Hb S/Δβ Arab</td>
<td>Hb S/E</td>
</tr>
<tr>
<td>Hb S/HPFH**</td>
<td></td>
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</tbody>
</table>

Notes:
- *This is inclusive of Hb S/β+, Hb S/β0, Hb S/ββ, HbS/γβ and Hb S/Lepore.
- **In general Hb S/HPFH is regarded as a milder condition than the other sickling conditions. It is the policy of the screening programme that follow up is offered in order to distinguish it from other more significant abnormalities. It is not possible at birth to differentiate with certainty between sickle cell anaemia (Hb SS), Hb S/β thalassaemia and Hb S with hereditary persistence of fetal haemoglobin (Hb S/HPFH), since all of these conditions produce only Hb F and
Hb S on analysis. For the purpose of this programme it is essential to detect and report all such cases as 'results consistent with sickle cell disease' without further detail in order to facilitate follow up and diagnostic testing.

- Since there are many Hb ‘D’ variants and characterisation of the variant may take time, it is recommended that all ‘D’ haemoglobins with the same analytical characteristics of D^Punjab (also called D^Los Angeles) are provisionally identified as this haemoglobin, the only clinically significant haemoglobin D variant. DNA analysis or mass spectrometry can then be used to elucidate the diagnosis.

Other clinically significant haemoglobinopathies likely to be detected by newborn screening

In addition to sickle cell diseases, there is another set of conditions presenting as beta thalassaemia major (including E/β^0 thalassaemia) which are likely to be detected by the screening programme and in which the patient can benefit from follow up. The UK National Screening Committee has agreed that, in line with other national screening programmes, clinically significant findings of conditions which are not part of the screening programme, but are detected by current screening methods, should always be reported to the relevant clinician to facilitate management of the consequences of such findings.

Notes:
- Little or no haemoglobin A on newborn screening is clinically significant since it may indicate little or no beta chain synthesis. The policy of the screening programme is that all cases where the apparent Hb A concentration is 1.5% or less of the total haemoglobin, the result should be reported as F only (or FE only if E is present) and followed up clinically.
- The newborn screening programme will not detect all cases that subsequently manifest as β thalassaemia major since some of these babies have an Hb A value greater than 1.5% at birth.
- Possible Hb E/β thalassaemia should be reported by newborn screening laboratories as many of these children will become transfusion dependent or have thalassaemia intermedia. Note that homozygous Hb EE and Hb E/β^0 thalassaemia will look identical on the initial screening test and will need to be differentiated.

Carriers and other ‘clinically benign’ haemoglobinopathies likely to be detected by newborn screening

Whilst the purpose of this programme is to detect infants with sickle cell disease, the analytical procedures currently utilised also detect homozygotes and compound heterozygotes for a variety of other haemoglobinopathies, as well as carriers of Hb S and the other common haemoglobin variants, (C, D^Punjab, O^Arab and E), and some of the rarer variants. Results of infants who are found to be homozygotes or compound heterozygotes for a common haemoglobin variant will be reported and follow up counselling offered. These are shown in the Table 2 below as the common clinically benign conditions.

Table 2: Generally clinically benign haemoglobinopathies

<table>
<thead>
<tr>
<th>Hb CC and C/β thalassaemia</th>
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<tbody>
<tr>
<td>Hb DD and D/β thalassaemia</td>
</tr>
<tr>
<td>Hb CD</td>
</tr>
<tr>
<td>Hb CE</td>
</tr>
<tr>
<td>Hb DE</td>
</tr>
<tr>
<td>Hb EE</td>
</tr>
</tbody>
</table>

Additionally, a small number of other variants may be detected using the present analytical procedures. These may not be immediately identifiable using the screening techniques and most will be benign. The number of neonates with one of these other variants is likely to be small. It is national policy that variants other than S, C, D, E and O^Arab should not be reported but the following
specimens should still be sent for second line testing:

1. Samples with 1.5% Hb A or less.
2. Samples with variants (peaks) more positively charged that Hb A, i.e. eluting after Hb A by HPLC and located to the right of Hb A on capillary electrophoresis.

This policy is designed to ensure the following:

1. Samples with little or no normal adult haemoglobin (Hb A) have the result confirmed before reporting.
2. Hb S (or one of the designated haemoglobins) is not missed even if it falls outside the predefined analytical windows.
3. Hb OArab, which has no defined analytical window, will still be identified.

The recommended wording for reporting the results for these mainly clinically benign variants, either homozygous, compound heterozygous or carriers, is given in Table 4 ‘Reporting newborn sickle cell screening results’ which uses the newborn status code 04 ‘Condition not suspected’. For any other report, the recommended wording should state that ‘haemoglobins S, C, Dpunjab, E and OArab have not been detected. Note that carriers of β thalassaemia and Hb Lepore are not detected by the techniques used for newborn screening’.

**Newborn sample requirements**

The same dried blood spot card is used for sickle screening as for the other newborn screening programmes. For the complete and proper processing of the specimen, four good quality spots are required (newbornbloodspot.screening.nhs.uk/guidelines).

Ideally, the sample should be taken at 5-8 days after birth (with the day of birth counted as day 0) and despatched to the newborn screening laboratory within 24 hrs of collection. Due to the nature of dried blood spots, there is deterioration of the blood sample from the time it is taken, as oxidation of the haemoglobin occurs resulting in methaemoglobin formation. This degradation is likely to be greater at higher temperatures, but in normal circumstances should not prevent analysis using the techniques described below. In occasional cases where there has been a delay in the card being sent to the laboratory or if it has been kept in unsuitable conditions, excessive oxidation may occur rendering the sample unsatisfactory for analysis.

In order for the sample to be processed satisfactorily and to facilitate patient follow up, it is essential that the dried blood spot card is completed fully:

- Adequate demographics for the infant and the mother, including the baby's NHS number and the mother's NHS number and place of delivery if available.
- Information regarding blood transfusion prior to sampling is important to avoid the error of analysing the haemoglobin of transfused red cells.
- The gestational age of the infant.
- Rank, if a multiple birth.
- Information about the family origin of the parents and the mother's antenatal screening test results is needed to help interpret the results and for quality assurance purposes.

**Pregnancies known to be at a 1 in 4 risk of a clinically significant haemoglobinopathy**

It is recognised that couples known to be at high risk (1 in 4) of having a baby with sickle cell disease or β thalassaemia major might wish to know the result for their child before the normal time for reporting the result from the bloodspot. To be a known ‘1 in 4’ high risk pregnancy, the haemoglobin results must be known for both parents. Local clinical practice policies should be in place for the parents, if they request it, to have a liquid capillary blood specimen (not cord blood) taken from the baby for analysis soon after birth. This is not part of the screening pathway and should be considered as an aspect of parental choice. The specimen should be taken in a clinical setting, rather than by the screening midwife.

This blood specimen should be sent to a specialist laboratory, which has expertise in haemoglobinopathy analysis in the newborn period. Not all haematology laboratories have expertise in analysing and interpreting results
on newborn babies. The screening programme has published Best Practice Guidelines for those maternity services where laboratories are requested to analyse newborn liquid capillary blood specimens from these known high risk pregnancies (see Appendix 5).

The fact that such a specimen has been taken should be noted by the midwife on the newborn screening bloodspot card. The screening laboratory will undertake the routine screen as usual and the test will act as a ‘failsafe’ and quality check. It is imperative that the test result is given to the parents as soon as possible to decrease their natural anxiety about the status of their child. Testing of the liquid specimen should be seen as a parallel test to the screening specimen and not a substitute.

Risks associated with babies who have had a transfusion containing red cells

The presence of transfused red cells in the neonate will interfere with the interpretation of the results from the haemoglobin analysis of the bloodspot and possibly invalidate the results. A small number of babies are transfused in utero but for babies transfused post-delivery it should be policy in all neonatal units to take a bloodspot specimen for sickle cell screening prior to giving a transfusion (NHS Screening Programmes: Guidelines for Newborn Blood Spot Screening, February 2012). A technique of analysing DNA extracted from the white cells from the bloodspot has been developed to overcome the complications caused by the presence of transfused red cells on the bloodspot card. At present this service is continuing to be funded by the National Screening Programme and is provided by Kings College Hospital and Sheffield Children’s Hospital. Arrangements for national screening programmes in England are under development. **It must be emphasised that this service does not eliminate the need to take a pre-transfusion specimen.**

The DNA test will detect the presence of the sickle globin gene and is able to differentiate between babies with only the sickle gene present: those with the sickle gene and another globin gene (either a normal beta gene or a beta gene with another variant); and those with no sickle gene present. All babies in whom the sickle gene is detected should be referred for clinical follow up. This test does not confirm the identity of the non-sickle haemoglobin and if the parents are known to be at risk of another haemoglobinopathy, they may wish for further standard haematology tests to be carried out on their baby. This is not considered part of the newborn screening programme and should be initiated in a clinical setting. However, it should be noted that testing using techniques other than DNA should not normally be undertaken until at least four months after the last transfusion.

**Percentages of Hb A in untransfused babies at different gestational ages**

To provide guidance about the expected levels of adult haemoglobin (Hb A) in newborn babies and babies up to one year of age, the following graphs (Figures 1 and 2) have been constructed from data provided by the newborn screening laboratory of Birmingham Children’s Hospital, which uses a BioRad VNBS analyser with valley to valley integration. The data on newborn babies is derived from 30,000 measurements and is presented as a percentile chart. The data on older babies from one month to one year is derived from 89 measurements and is therefore less statistically robust.

The purpose of the graphs is to provide guidance about the expected percentage of Hb A that is seen in babies born from 23 weeks gestation up to 1 year of age. Increased percentages of Hb A can be found following transfusion and the graphs may help to determine if the amount of Hb A is appropriate for the age of the baby. The primary way in which the transfused baby should be identified is the information provided by the healthcare professional who completes the bloodspot card. However, this data field on the card is not always completed. It is anticipated that the newborn screening laboratories can use the information provided in these graphs to reduce unnecessary referrals for DNA testing, especially in older babies. This is important as the failsafe screening test for sickle haemoglobin on transfused babies using DNA methods should only be used when a baby has received a transfusion and no pre-transfusion sample has been taken.
Sample analysis

Newborn screening for sickle cell disease using haemoglobin eluted from dried blood spots can be reliably undertaken using a primary screen to detect the different haemoglobin fractions present. In the case of suspected abnormality, a second-line test on the same specimen using different methodology is necessary to validate the initial findings. Using this approach, the identification of haemoglobin variants obtained is sufficiently accurate for clinical purposes. It is important to note that unequivocal identification of haemoglobin variants can only be achieved by either protein sequence analysis (e.g. using mass spectrometry) or analysis of DNA extracted from blood. It is also important in this screening programme to realise that occasionally the presumptive identification of a haemoglobin variant using screening methods is incorrect, since some variants give exactly the same results using current screening techniques. Screening is not a diagnostic service and no screening programme has a diagnostic sensitivity and specificity of 100%. Haemoglobins S, C, D (including D^Punjab), E and O^Arab should be detected reliably. With any Hb variant, misclassification should be minimal with proper use of the procedures documented below. However, it is important that reports are carefully worded in order to avoid misleading colleagues and parents as to exactly what has been achieved from the screen (see guidance on reporting of results - page 13).

Acceptable analytical protocols

The analytical procedures employed must be capable of detecting all the common clinically significant haemoglobin variants, i.e. 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 (75%) with approximately 25% Hb A and small quantities of acetylated Hb F and sometimes 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.

Three types of analysis are in use for newborn screening for sickle cell disease using dried blood spot samples: high performance liquid chromatography (HPLC); isoelectric focusing (IEF); and capillary electrophoresis (CE). Tandem mass spectrometry has also been shown to be suitable for newborn screening and its use in England is under review. These
methods are suitable for first line screening, with an alternative procedure using a different principle being used for second line testing to validate the apparent identity of the variant. It is recommended that all haemoglobin separations produced (i.e. gels and chromatograms) are initially read by the analyst undertaking the procedure, and then checked by a second person. It is unwise to rely on transmitted data from automated equipment without checking the haemoglobin separations, due to the possibility of misclassification of fractions. Since the pre-analytical procedures are easy to perform and automated equipment is relatively easy to operate, staff who are skilled and trained in the procedures but not necessarily formally qualified scientists, can undertake this work. However, it is recommended that reading the separations and chromatograms, and the interpretation and checking of all results and reports, is undertaken by suitably trained, HCPC-registered laboratory staff.

High performance liquid chromatography (HPLC)

High performance liquid chromatography utilizes an ion exchange resin, held in a column cartridge, in conjunction with a buffer gradient. As the ionic strength and/or pH of the buffer changes, so certain haemoglobins are eluted from the column and the presence of haemoglobin is detected using a spectrophotometric technique. The time from injection to the point at which the haemoglobin fraction elutes is known as the retention time of the haemoglobin and is a reproducible measurement for a particular column, buffer, exchange resin and temperature. However, it is quite common for different haemoglobins to elute at the same retention time. Thus the retention time is not a unique identifier. Hb F is eluted separately from Hb A. Haemoglobins S, C, D, E also have separate retention times and characteristic chromatographic profiles. In addition, the relative proportions of the different haemoglobins are recorded. It is therefore possible to detect the difference between carriers and affected infants and also to differentiate some types of compound heterozygosity of Hb S with β+ thalassaemia.

Isoelectric focusing (IEF)

IEF gives good separation of Hb F from Hb A and variant haemoglobins S, C, D<sub>Punjab</sub>, E and O<sub>Arab</sub>. The separation of different haemoglobins is accomplished through application of a haemoglobin sample onto a precast agarose gel containing ampholytes at pH 6-8. Ampholytes are low molecular weight amphoteric molecules with varying isoelectric points (pl). When an electric current is applied, these molecules migrate through the gel to their isoelectric points forming a stable pH gradient. The haemoglobin variants also migrate through the gel until they reach the point at which their pl equals the corresponding pH of the gel. At this point, the net charges on the variants are zero and migration ceases. The electric field counteracts diffusion and the haemoglobin variants form discrete thin bands. IEF can be semi-automated, rendering the technique suitable for screening large numbers of samples.

Capillary Electrophoresis (CE)

Capillary electrophoresis utilises a combination of ion migration and electro-osmotic flow to separate protein molecules. When a voltage is applied across the capillary tube filled with an electrolyte solution, the solution begins to move towards one of the electrodes due to electro-osmotic flow. This drives the bulk flow of materials past the detector in the same way that a pump pushes the liquid in HPLC. The haemoglobin molecules move towards the detector at different speeds depending on their ionic charge and electrophoretic mobility. Both electro-osmotic flow and electrophoretic mobility are occurring at the same time, working in opposite directions to provide greater resolution. This method of separation should not be confused with simple electrophoretic mobility as seen in cellulose acetate electrophoresis. Combining electro-osmotic flow and electrophoretic mobility is a separate phenomenon and is exploited in CE for maximum separation power. Even so, it is quite common for different haemoglobins to migrate at the same rate and appear at the same position. Thus position is not a unique identifier. Hb F is separated from Hb A. Haemoglobins S, C, D, and E also have different mobility rates and characteristic profiles. In addition, the relative
proportions of the different haemoglobins are recorded. It is therefore possible to detect the difference between carriers and affected infants and also to differentiate some types of compound heterozygosity of Hb S with β+ thalassaemia.

General analytical considerations in newborn screening

Users should be aware that the Laboratory Handbook highlights common analytical and diagnostic issues but every laboratory should follow the principles of good laboratory practice, including satisfying themselves that they understand the capabilities and limitations of their chosen technique. The equipment and protocol chosen must fulfil the requirements of the screening programme and demonstrate suitable performance on EQA.

The use of rules to screen samples for further action/reporting or the use of post analytical data analysis algorithms are not recommended without software quality control procedures, including regular process audit to ensure that quality is not compromised. It is essential that the process is risk assessed and that there are failsafe mechanisms in place. Raw data including chromatograms must always be reviewed and any post analytical procedures including algorithms, must be fully documented and traceable to ensure consistency of quality should staffing change over time.

The application of HPLC and IEF for newborn screening has a disadvantage in that the process also separates the normally occurring adducted fractions, i.e. acetylated Hb F (Hb F₁) and glycated haemoglobins, which can make interpretation difficult. The retention times and migration patterns of different haemoglobin variants are not unique and thus the results obtained can only be regarded as provisionally identifying the variant(s) concerned.

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. This is usually achieved by the use of retention time markers.

If IEF is used, then control haemoglobins must be run with each plate. Care should be taken to ensure that there is clear delineation between the bands for adjacent specimens. This can be achieved by firmly blotting the gel and blotting again after the template has been added before the addition of the sample.

If CE is used, appropriate control material must be used to ensure optimal analytical performance. Optical density (OD) levels greater than 0.07 and the presence of sufficient Hb F are required to determine the migration position and thus permit ‘zoning’ and a provisional identification of haemoglobins present in the sample. If failure to zone is due to low OD, this is usually related to the amount of haemoglobin in the sample. This should be corrected by increasing the spot to diluent ratio and allowing a longer elution time. Extreme care is needed if extraneous haemoglobin is added to a clinical sample to allow zoning. The addition of haemoglobins which were not present in the initial sample will make the chromatogram more difficult to interpret and may lead to the misinterpretation of the results. Such modified samples should be analysed with a unique identifier distinguishable from both the original specimen and from any other clinical sample. Experiments have shown that denatured Hb F (acetylated F) which increases with age of the sample, appears in zone N9 but can appear on the borders of zone N10. When only a small amount of Hb F is present the two may overlap, falsely increasing the measured level of Hb A. Caution is needed in interpreting such results.

Interpretation of results

Results of sickle cell screening are interpreted according to the different haemoglobin fractions present, which in unaffected infants are Hb F (as the major fraction) and Hb A. If the results of the first line screen show the presence of any abnormal fraction, second line testing must be considered according to the guidelines on page 26. If the concentration of Hb A on the bloodspot is abnormally high or comprises all of the haemoglobin present,
then the possibility of a transfusion prior to
the blood being taken. Contamination of the
bloodspot with adult blood as a result of poor
practice should be considered. In very rare
cases the baby may have γ thalassaemia which
can mimic a blood transfusion or
contamination with adult blood. In these
cases, further investigation must take place.

**Unaffected:** Results from unaffected infants
show the presence of Hb F and Hb A. It is
usual in a term infant for the percentage of
Hb A to be between 5 and 30%. This figure
varies according to gestation and the
laboratory must consider gestational age
when requesting repeat sampling. See Figures
1 and 2.

**Sickle cell disease:** Results from infants with
sickle cell disease show the presence of Hb F
and Hb S in the absence of Hb A (i.e. FS); or
Hb F and Hb S with another haemoglobin
variant (e.g. FSC); or Hb F, Hb S and Hb A
where the quantity of Hb S is greater than Hb
A (Hb S/β+ thalassaemia). In cases of sickle
cell disease (F+S only) the quantity of Hb S
found is usually between 4 and 10% for a
term baby and less in a premature baby. With
some HPLC systems a very small peak elutes in
the Hb A0 window and so a baby with Hb SS
may appear to have Hb S/β+ thalassaemia, but
with experienced personnel checking the
chromatograms, this is not usually a problem.
Expected results from infants with sickle cell
disease are shown in Table 3.

**Hb S/β thalassaemia:** Caution should be
exercised in the interpretation of results where
the amount of Hb S is greater than Hb A, as
the cause may be Hb S/β+ thalassaemia. If Hb
S is greater than Hb A, check the parents’
results if these are available. If the parents’
results are not available, the Hb S would
normally be expected to be greater than
120% of the HbA result for a diagnosis of Hb
S/β thalassaemia to be made. Again, the
actual quantities of Hb A and Hb S found will
vary according to gestational age.

**Table 3: Newborn screening results for
sickle cell disease**

<table>
<thead>
<tr>
<th>Newborn screening results</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS</td>
<td>Hb SS</td>
</tr>
<tr>
<td>FS</td>
<td>Hb S/β0 thalassaemia</td>
</tr>
<tr>
<td>FS</td>
<td>Hb S/δβ thalassaemia</td>
</tr>
<tr>
<td>FS</td>
<td>Hb S/HPFH</td>
</tr>
<tr>
<td>FSA or FS</td>
<td>Hb S/β+ thalassaemia</td>
</tr>
<tr>
<td>FSC</td>
<td>Hb SC</td>
</tr>
<tr>
<td>FSD</td>
<td>Hb S/D\text{Punjab}</td>
</tr>
<tr>
<td>FSE</td>
<td>Hb S/E</td>
</tr>
<tr>
<td>FSO\text{Arab}</td>
<td>Hb S/O\text{Arab}</td>
</tr>
</tbody>
</table>

**Sickle cell carrier:** Results from infants who
are sickle cell carriers will show the presence
of Hb F, Hb A and Hb S. The quantity of Hb A
should exceed the quantity of Hb S. In some
cases the amounts of Hb S and Hb A are
almost equal.

**Heterozygotes for other haemoglobin
variants:** Results from infants who are carriers
for other haemoglobin variants will show the
presence of Hb F and Hb A and the
haemoglobin variant. Again, the quantity of
the variant is usually less than the quantity of
the Hb A and the exact quantities will vary
with gestational age.

**Homozygotes or compound heterozygotes
for non-sickling conditions:** Results from
infants who are homozygous or compound
heterozygotes for conditions other than sickle
cell disease, will show the presence of Hb F
and the haemoglobin variant(s) only. No Hb A
will be detected. Results from some
automated analysers may be misleading in
that an apparent small Hb A, peak may be
present, but this should not be a problem with
experienced personnel checking the
chromatograms.

**Beta thalassaemia major:** Results from a
child with severe β thalassaemia will usually
have only Hb F present and no Hb A. The
policy of the screening programme is that all
cases where the apparent Hb A concentration
is 1.5% or less of the total haemoglobin, the result should be reported as F only and followed up. In a small survey of newborn babies with confirmed β thalassaemia major, 81% had apparent Hb A present in the screening specimen. Therefore caution should be exercised in cases where there appears to be a small amount of Hb A. Additionally many different mutations give rise to severe β thalassaemia and some of these may result in the presence of very small amounts of Hb A. DNA analysis is required to elucidate the diagnosis.

In theory, antenatal screening should identify all ‘at risk’ couples for babies with serious thalassaemias (assuming that the father has been tested where appropriate), and these babies should be specifically diagnosed at birth by DNA analysis in accordance with good clinical practice. If babies are not identified in pregnancy, the newborn screening programme will act as a failsafe for the antenatal screening programme as well as an audit of effectiveness.

It is important to emphasise that not all thalassaemias will be detected by the newborn screening programme. The majority of babies with β thalassaemia major will be detected by a very low Hb A (1.5% or less) at birth, but for the other thalassaemias there are no reliable indicators that will be detected using currently available screening methods.

Risk assessment for newborn screening

Conditions that are not likely to be detected using proposed screening protocols:

As this is a screening programme rather than a diagnostic service, the following conditions will not be detected:

- β thalassaemia major or intermedia with an HbA value greater than 1.5%
- β thalassaemia carriers
- Hb H disease and α thalassaemia carriers
- Hb S/β thalassaemia with a very high expression of Hb A
- rare clinically significant haemoglobins (e.g. high affinity haemoglobins, unstable haemoglobins and methaemoglobin forming variants)
- rare haemoglobin variants that are clinically significant in the presence of Hb S
- undeclared transfused babies whose Hb A level is below the action values shown in figures 1 and 2
- any significant variant which is misinterpreted as a result of an undeclared blood transfusion, in particular the risk that an Hb FS may be reported as Hb FAS.

Reporting results for the newborn screening programme

The following guidelines, on reporting analytical data and the genetic implications (conclusions) of that data plus Table 4, should provide sufficient information to allow all unaffected and over 95% of abnormal cases to be reported in a standardised manner.

Appendix 2 gives more detail on the status codes required for use when reporting newborn screening results to Child Health systems and for administrative use. These codes are used to support the transfer of information between the respective computer systems and they are not intended to be used for clinical purposes. The codes are designed to be generic for all of the conditions that are screened for in the newborn bloodspot screening programme. Examples of the different types of sickle cell phenotypes that fit into the different report codes are given in Table 4.

If the newborn baby has had a blood transfusion and any of the transfused red cells are still present, misleading data and conclusions may result. This includes ‘in utero’ transfusions a neonate may have received. It is therefore essential that clinicians realise this fact and consideration should be given to having a footnote on all haemoglobinopathy results, such as ‘Result only valid if not transfused’, or something similar, when it is practical to do so.

General notes on reporting

1. The sample date must be given. This is essential if a baby has had a recent blood transfusion.

2. Analytical fact must be separated from interpretative opinion. The factual results should be given and then a clear
conclusion, which may include recommendations.

3 The haemoglobins present should be reported in the order of the greatest to the least percentage.

4 Since it improves clarity, the conclusion should always be given in full text, for example: ‘Results consistent with sickle cell carrier’ is the conclusion for Hb FAS or ‘Results consistent with sickle cell disease’ for an analytical result of Hb FS. Since it is not possible to have at least three different techniques relying on different physicochemical properties when undertaking newborn screening from bloodspot cards, the conclusion should be prefaced by ‘Results consistent with’.

5 Both the analytical facts and the conclusion must be clear. This should ensure that the report will lead to the action that is considered necessary and will not lead to inappropriate worry.

6 The reports and templates used must be clearly presented and laid out so that misinterpretation is avoided.
Table 4 - Reporting newborn sickle cell screening results Note: ‘Thalassaemia’ = β⁰, β⁺, δβ, γδβ and Hb Lepore as appropriate

<table>
<thead>
<tr>
<th>Analytical results (Hb written in order of %)</th>
<th>Diagnostic possibilities</th>
<th>Suggested report format</th>
<th>Suggested status code for Child Health reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>SICKLE CELL DISEASE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| FS Fetal and sickle haemoglobin             | • Sickle cell anaemia (81%)  
• Sickle cell - β thalassaemia (17%)  
• Sickle cell - HPFH (2%)  
(Figures in parenthesis indicate the relative prevalence of each condition in London*) | • Results consistent with sickle cell disease  
• Follow up referral required  
• Actual genotype will require further investigation | 8 |
| FSA Fetal haemoglobin, sickle haemoglobin and a small amount of adult haemoglobin | • Sickle cell - β thalassaemia  
• Transfusion  
• Where larger amounts of Hb A (but still less than Hb S) are present the final result maybe a sickle cell carrier but clinical follow up of these cases is always necessary | • Results consistent with sickle cell disease  
• Result valid only if not transfused  
• follow up referral required  
• Actual genotype will require further investigation | 8 |
| FSC Fetal haemoglobin, sickle haemoglobin and haemoglobin C | • Haemoglobin SC disease | • Results consistent with Hb SC disease  
• follow up referral required | 8 |
| FSD Fetal haemoglobin, sickle haemoglobin and haemoglobin D | • Haemoglobin SD disease | • Results consistent with Hb SD disease  
• follow up referral required | 8 |
| FSE Fetal haemoglobin, sickle haemoglobin and haemoglobin E | • Haemoglobin SE disease; generally a milder form of sickle cell disease | • Results consistent with Hb SE disease  
• follow up referral required | 8 |
| FSOArab Fetal haemoglobin, sickle haemoglobin and haemoglobin OArab | • Haemoglobin SOArab disease | • Results consistent with Hb SOArab disease  
• follow up referral required | 8 |

* The figures for the birth prevalence of the sickle cell disease were obtained from 146 children identified by newborn screening in London and confirmed by parental results.
<table>
<thead>
<tr>
<th>Analytical results (Hb written in order of %)</th>
<th>Diagnostic possibilities</th>
<th>Suggested report format</th>
<th>Suggested status code for Child Health reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OTHER POTENTIALLY CLINICALLY SIGNIFICANT DISORDERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>F only</strong>&lt;br&gt;Fetal haemoglobin (or with adult haemoglobin 1.5% or less)</td>
<td>- Possible - β thalassaemia major&lt;br&gt;- Prematurity&lt;br&gt;- Homozygous HPFH&lt;br&gt;- HPFH with β thalassaemia</td>
<td>- Only fetal haemoglobin detected&lt;br&gt;- follow up referral required</td>
<td>7</td>
</tr>
<tr>
<td><strong>FE and FEA</strong>&lt;br&gt;Fetal haemoglobin and haemoglobin E with possibly an adult haemoglobin 1.5% or less</td>
<td>- Haemoglobin E/β thalassaemia; a form of thalassaemia that may cause transfusion dependence&lt;br&gt;- Haemoglobin E disease; a mild form of haemolytic anaemia&lt;br&gt;- Haemoglobin E with HPFH</td>
<td>- Possible homozygous Hb E or Hb E/β thalassaemia&lt;br&gt;- follow up referral required</td>
<td>7</td>
</tr>
<tr>
<td><strong>BENIGN CONDITIONS AND CARRIERS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FC or FD or FO_{Arab}</strong>&lt;br&gt;Fetal haemoglobin and haemoglobin C or haemoglobin D or haemoglobin O_{Arab}</td>
<td>- Homozygous for Hb C or D or O_{Arab}&lt;br&gt;- Compound heterozygote for Hb C or D or O_{Arab} and β thalassaemia or HPFH</td>
<td>- Possible homozygous Hb C or D or O_{Arab}&lt;br&gt;- Possible compound heterozygous Hb C or D or O_{Arab} and β thalassaemia</td>
<td>7</td>
</tr>
<tr>
<td><strong>FCA or FDA or FO_{Arab}A</strong>&lt;br&gt;Fetal haemoglobin, adult haemoglobin and haemoglobin C or D or E</td>
<td>- Compound heterozygote for Hb C or D or O_{Arab} and β⁺ thalassaemia</td>
<td>- Possible compound heterozygous Hb C or D or O_{Arab} and β⁺ thalassaemia&lt;br&gt;- Valid if not transfused</td>
<td>7</td>
</tr>
<tr>
<td>Analytical results (Hb written in order of %)</td>
<td>Diagnostic possibilities</td>
<td>Suggested report format</td>
<td>Suggested status code for Child Health reporting</td>
</tr>
<tr>
<td>---------------------------------------------</td>
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<td>-----------------------------------------------</td>
</tr>
</tbody>
</table>
| **FSV**
Fetal haemoglobin, sickle haemoglobin and an unidentified variant haemoglobin | • Conditions phenotypically similar to sickle cell carrier.
• Usually clinically benign but genetically significant (see newborn risk assessment) | • Results consistent with sickle cell carrier | 5 |
| **FAS**
Fetal haemoglobin, adult haemoglobin and haemoglobin S | • Sickle cell carrier; clinically benign but genetically significant | • Results consistent with sickle cell carrier
• Valid if not transfused | 5 |
| **FAC or FAD or FAE or FAO\textsubscript{Arab}**
Fetal haemoglobin, adult haemoglobin and haemoglobin C or D or E or O\textsubscript{Arab} | • Hb C carrier or Hb D carrier or Hb E carrier or Hb O\textsubscript{Arab} carrier; clinically benign but genetically significant | • Results consistent with Hb C carrier or Hb D carrier or Hb E carrier or Hb O\textsubscript{Arab} carrier
• Valid if not transfused | 6 |
| **FV, FVA and FAV**
Fetal haemoglobin, with or without adult haemoglobin and a haemoglobin variant (not S, C, D, E or O\textsubscript{Arab}) | • Most likely clinically insignificant haemoglobin but may be genetically significant | • Haemoglobins S, C, D, E and O\textsubscript{Arab} not detected
• Note – Carriers of β thalassaemia and Hb Lepore cannot be excluded at this age
• Valid if not transfused (exclude this if result is FV) | 4 |
| **FA**
Fetal and adult haemoglobin | • No haemoglobin variant detected | • Haemoglobins S, C, D, E and O\textsubscript{Arab} not detected
• Note – Carriers of β thalassaemia and Hb Lepore cannot be excluded at this age
• Valid if not transfused | 4 |
Issuing laboratory reports

The parents and GP should be informed of all the outcomes of screening. The approach adopted should follow general guidance from the UK Newborn Bloodspot Programme Centre.

Laboratories are responsible for sending all screening results to Child Health departments or their equivalent in a timely manner. See Appendix 2 for the relevant status codes. This information will be used to assess coverage of the screening programme and to provide a mechanism for reporting ‘normal’ results to parents and other healthcare professionals. Presumptive positive results should be reported immediately by the laboratory to the designated healthcare professional.

Annual data returns

An annual data return will be required from all newborn screening laboratories using the template that has been developed in conjunction with the UK Newborn Bloodspot Programme Centre. This requests data on sickle cell screening as well as the other screening tests performed on the newborn bloodspot. See newbornbloodspot.screening.nhs.uk/datacollection Data also needs to be collated and submitted in relation to the relevant Key Performance Indicator (KPI) returns for the newborn screening programme.

Action required for particular categories of results

Infants with sickle cell disease

Results should be sent by the laboratory as a matter of urgency (fax/electronically, etc) to the designated healthcare professional and confirmation of receipt documented. Parents should be informed by personal contact. Copies of all reports should be sent to the GP and health visitor. These babies should also be referred to the appropriate specialist network lead (a list of these can be found in Appendix 11) and to the newborn outcomes project.

Infants heterozygous for haemoglobins S, C, D, E or OArab

Results should be sent by the laboratory to the designated healthcare professional and confirmation of receipt documented. Parents and GPs should be informed by a locally agreed mechanism.

Infants with no abnormality detected or haemoglobin variants other than S, C, D, E or OArab

Results should be provided in written form by the child health department or equivalent for the parents of the child and the child’s GP. The recommended wording for reporting the results for these infants with no abnormality detected or haemoglobin variants other than S, C, D, E or OArab uses the newborn status code 04 ‘Condition not suspected’. The recommended wording should state that ‘haemoglobins S, C, D, E and OArab have not been detected. Note that carriers of β thalassaemia and Hb Lepore are not detected by the techniques used for newborn screening’.

Premature Infants

Hb A is normally detectable by 30 weeks gestation and is sometimes detected by 24 weeks. Results from premature infants should be interpreted with caution. Premature infants who show Hb A of 1.5% or less need repeat testing to check for the presence of sickle cell disease or β thalassaemia major.

Quality Assurance and Improvement

In August 2008, the UK Newborn Screening Programme Centre published ‘Standards and Guidelines for Newborn Blood Spot Screening’ (newbornbloodspot.screening.nhs.uk/standards). The nine standards it contains are
based on the original 6 standards published in 2005 and aim to drive the improvement in the quality of the bloodspot sample and the timeliness of screening. The ‘acceptable/achievable standards’ set out the expected level of performance to deliver an acceptable level of quality. The ‘developmental standards’ depict a level of performance that delivers enhanced quality. The standards are accompanied by a number of best practice guidelines, which should be followed to deliver high quality screening processes and to meet the standards.

Minimum criteria (standards) for laboratories undertaking newborn screening

The non-cancer screening programmes are developing a quality assurance framework that will operate across the antenatal and newborn programmes. This framework covers the screening pathway from offer of testing through to entry into care, as well as the user experience, equity, governance and commissioning. This process aims to ensure there are no gaps or duplications with existing processes such as CPA and EQA assessments. There are also programme specific quality assurance processes. The quality improvement acceptable and achievable standards are given in detail in the NHS Sickle cell and Thalassaemia Screening Programme: Standards for the linked antenatal and newborn screening programme sct.screening.nhs.uk/cms.php?folder=2493). These are referred to in the text as standard references.

1. The laboratory must be appropriately accredited with a nationally approved accreditation scheme such as Clinical Pathology Accreditation UK (Ltd), now formally part of the United Kingdom Accreditation Service (UKAS).

2. The workload of the newborn screening laboratory should exceed 25,000 specimens per year (ideally 50,000), to give appropriate economies of scale and confidence in the interpretation of abnormal results.

3. There must be a senior member of the laboratory staff with haematological expertise at medical consultant or clinical scientist/biomedical scientist consultant level responsible for the haemoglobinopathy screening section of the service, with defined lines of accountability and authority for all laboratory aspects of the service. The team must also include members with relevant haematological/haemoglobinopathy expertise.

4. The initial screening test must be performed using high performance liquid chromatography (HPLC), isoelectric focusing (IEF), capillary electrophoresis (CE) or a method giving comparable results, with a confirmatory test for the positive results being performed by repunching the original blood spot and using a different technique from the initial screening test. For newborn sickle cell screening, the acceptable standards for sensitivity of the screening test (standard reference NO2i) are:
   • 99% detection for Hb SS
   • 98% detection for Hb SC and
   • 95% detection for other conditions.

5. Screening laboratories must have the IT capacity to support standard reporting and audit requirements of programme. In particular the recommendations made in the ‘Reporting results for the newborn screening programme’ should be followed. Screening laboratories must use the appropriate screening status codes when interfacing with the child health record systems (standard reference NS2). The recommended data fields for the annual return required for monitoring and audit purposes must also be collected.

6. The acceptable standards for timeliness of reporting are:
   • 95% of screen negative results including haemoglobinopathy carriers available by 6 weeks of age (standard reference NP2i)
   • 90% of sickle cell disease results available by 4 weeks of age (standard reference NP3).

7. There must be a documented risk management policy for the laboratory aspects of the haemoglobinopathy screening service. This should include a risk assessment which describes the steps in the testing protocol where mistakes could
occur and the procedures that have been implemented to minimise the risk of the mistake occurring. Where appropriate this should include a policy for any samples sent away for further analysis. It should also include documented procedures for the management and reporting of incidents.

8 The laboratory must participate in an accredited External Quality Assessment Scheme (EQAS), appropriate for newborn screening, e.g. UK NEQAS, and must be able to able to demonstrate satisfactory performance as defined by the criteria specified by the EQA scheme organisers. It is expected that laboratories will participate specifically in UK NEQAS and will agree to share performance information with the Programme Centre.

9 Appropriate internal quality control procedures must be undertaken and documented, e.g. recording of reagent lot numbers, recording of turnaround time for reports, results of internal quality control specimens.

10 The laboratory must participate in audit at local and regional level, with the results of the newborn screening programme being published in an annual report of process and outcome to ensure the laboratory is meeting the Programme’s aims and objectives (standard reference NS2). There must be links established with the antenatal screening laboratories to audit the effectiveness of both arms of the national screening programme.

11 Screening laboratories must have failsafe arrangements in place. This includes checking all samples have been received, and all results are received and acted upon (both positive and negative) in a timely manner. This must include a system to record information received from maternity units about at risk couples and links made to previous screening results. All screen-positive results must be reviewed regularly.

12 The laboratory must be willing to release information on screening performance, including incidents, to any appropriate monitoring group of the National Screening Committee and the NHS Sickle Cell and Thalassaemia Screening Programme Centre, and be open to peer review visits and inspection by the commissioners or their representatives at any reasonable time, by mutual agreement (standard reference NS2).

13 There must be regular meetings held at least quarterly to review accountability and responsibility and screen positive results across the linked programme, including failsafe (standard reference LS1 and standard reference AS1 - acceptable standard 80% of meetings to be held at least quarterly).

14 Laboratories will be expected to comply with the ‘Code of Practice for the Retention and Storage of Residual Spots’ published in the Policies and Standards of the UK Newborn Screening Programme Centre.

15 When reports received from third parties are transcribed into internal laboratory information systems, a full and exact copy of the report must be made. It is not acceptable to summarise or leave out information. The transcribed report should always be checked by an appropriate second person to ensure accuracy of transcription. If laboratories have a system to scan a copy into the laboratory information system this would be a preferred alternative. A report or an exact copy/photocopy of the report should also be included in the patient’s notes and included when referrals are made for counselling or advice.

Lines of responsibility

The midwife is responsible for:

- informing the parent(s) or guardian of the reasons for testing
- providing relevant information to parent(s) or guardian
- offering the test, ensuring positive identification and collecting the dried blood spot sample ideally 5-8 days after birth, labelling and despatching the sample within 24 hours of collection
- ensuring that the bloodspot card has the baby’s NHS number for correct identification and to confirm coverage of the programme, and ideally the mother’s NHS number to allow linkage with antenatal records and screening results
• ensuring that the information relating to transfusion history is correctly completed
• obtaining informed consent and supplying written notification to the laboratory if the parents wish to opt out of testing
• taking repeat specimens when requested by the laboratory.

The screening laboratory is responsible for:
• documentation of the baby's demographics, specimen analysis and issuing of results within a timely manner after receipt of the sample
• reporting results requiring referral and follow up to the designated healthcare professional
• reporting all results to the relevant child health department
• reporting all affected babies to the newborn outcomes project at the NHS Sickle Cell and Thalassaemia Screening Programme Centre
• providing information on request to PND laboratories to enable completion of PND outcomes
• reporting all affected babies to the appropriate clinical network for follow up
• sharing data with antenatal and second line screening laboratories.

The Designated Healthcare Professional with relevant skills and training is responsible for:
• informing parents of the results and arranging clinical follow up of babies with confirmed or suspected sickle cell disease
• informing parents of the results and arranging clinical follow up of babies with other potentially clinically significant conditions
• ensuring that babies are not lost to clinical follow up before registration in a clinic
• providing information and counselling for the parents of babies who are carriers or have other benign conditions detected
• arranging repeat testing as indicated by the laboratory.

The Child Health Department is responsible for:
• checking that all newborn babies have been screened or that screening has been declined
• recording the results of screening in the child health information system
• disseminating the status code 4 (condition not suspected) results for all conditions to parents
• ensuring babies without results are identified and the responsible health care professional notified.

Clinical network arrangements
It is recommended that the screening laboratory is linked to the networks of clinical care. Each of these networks will have a recognised specialist haemoglobinopathy centre which will take a leadership responsibility across a defined region agreed with specialist commissioners. This leadership role will include identification of a named specialist clinician for every patient and the responsibility for the annual review of all affected infants. The development of the clinical care networks will be led by the Specialised Commissioners in conjunction with the specialist centres.

As described in the ‘Guide to effectively commissioning high quality sickle cell and thalassaemia services’ newborn screening laboratories report screen positive sickle cell results to local clinicians and to the specialist or network centre. This will act as a failsafe to ensure screen positive babies enter the care pathway. The specialist centre will have responsibility for checking that screen positive babies have been entered into the care pathway.

There is a set of required data collection items, which are described in Appendix 7 of the Sickle Cell disease in Childhood Standards and Guidelines for Clinical Care 2nd Ed. October 2010 (sct.screening.nhs.uk/getdata.php?id=11164). These are to support monitoring of newborn outcomes: in particular to assess mortality and morbidity of children affected by haemoglobinopathies up to age 5; the timely entry of affected babies/children into care; and a look back to the mother’s antenatal screening history. This document is currently
under revision and relevant stakeholders will be notified when it is available.

The scope of the responsibility of specialised commissioners is currently available on the Department of Health website; a specification for specialist services will be available towards the end of October 2012.

There is also now a National Definitions Set for Specialised Haemoglobinopathy Services (number 38) for care of those with sickle cell and thalassaemia (sct.screening.nhs.uk/news.php?id=10816).

Support services for timely follow up and treatment need to be in place and co-ordinated across the area covered by a programme, to ensure that the potential benefits of the programme are realised. Experience from the USA shows that the main reason for the failure of a screening programme in terms of clinical outcomes is failing to ensure that identified infants are registered in a programme of treatment and care or failing to identify that, having been registered, they are subsequently lost to follow up.

**Failsafe arrangements**

Each local area needs to have fail-safe arrangements in place, with designated individuals responsible from the relevant professional disciplines. These are distinct from the care pathway for individual users of the service and also from the responsibilities of individual professionals in following up particular actions for individual patients.

For the newborn programme this includes having a system to ensure that there is a review of screen positive infants and ensuring enrolment in appropriate follow up care, with a defined programme of treatment with a relevant local service provider and a recognised specialist unit as appropriate. Treatment should comply with *NHS Screening Programmes, Sickle Cell Disease in Childhood: Standards and Guidelines for Clinical Care, 2010.*

For both the newborn and the antenatal programmes (working together as a linked programme), this includes a requirement for a formal independent process, such as a regular audit meeting, for the review of all screen positive results and action taken to follow up screen positives cases. This should be undertaken on a regular basis at least annually, with specified individuals involved and clear accountability, to ensure that processes of care operate smoothly and in a timely manner.
Antenatal screening

The overall aim of the antenatal screening programme is to offer sickle cell and thalassaemia screening to all women and couples in a timely manner in pregnancy. The screening programme aims to facilitate informed choices regarding participation in the screening programme and provide help for those couples identified by screening as being at higher risk.

For all pregnant women presenting to maternity services in England, sickle cell and thalassaemia screening should be an integral part of their early antenatal care.

Testing women in subsequent pregnancies

There has been considerable debate about the need to screen women for sickle cell and thalassaemia multiple times if they have already been tested in a previous pregnancy or for some other reason. In each pregnancy, the woman should have a routine full blood count taken at the antenatal booking visit and MCH and other red cell indices should be reassessed. The recommended policy for sickle cell and thalassaemia screening is that women need not be tested again in the same or a subsequent pregnancy provided that:-

- there are two or more previous results from a reputable laboratory, preferably accredited by a body in the UK, which are consistent, unequivocal and well documented. These results must be interpreted in line with recommendations in this handbook.
- the red cell indices remain the same and can be used for a reliable interpretation. (If the MCH has been ≥27pg on two previous occasions but the routine blood count in this pregnancy shows the MCH <27pg, the woman need not be tested for thalassaemia.)
- the woman’s identification has three or more matching data items, e.g:-
  - name, date of birth, medical record number or NHS number
  - name, date of birth and address
  - medical record number, NHS number, date of birth and address (if woman confirms name change)
  - name, date of birth and haemoglobinopathy card.


If a previous result is being used then this fact must be recorded in the woman’s notes for the current pregnancy.

There are many recorded instances where the results do not match when the same patient is tested on two different occasions. The reasons can vary from errors in labelling of specimens and laboratory errors to deliberate swapping of identities and duplicate medical record numbers. There may also be due to legitimate technical reasons, especially inherent variability of measurements occurring around action values. For this reason it is expected that local policies be in place to set out the protocols that should be followed in each location to minimise such risks. These may vary depending on previous experience and the prevalence of such problems.

Testing the baby’s father if he has been tested previously

If the baby’s father has been tested for sickle cell and thalassaemia previously, the same protocol should be followed as for a previously tested mother. It would be wise to test for a second time to confirm the previous results and then he need not be tested again provided that:-

- there are two or more previous results from a reputable laboratory, preferably accredited by a body in the UK, which are consistent, unequivocal and well documented.
- the red cell indices remain the same and can be used for a reliable interpretation or have previously been normal on two occasions.
- his identification has three or more matching data items, e.g:
  - name, date of birth, medical record number or NHS number
  - name, date of birth and address
  - name, date of birth and haemoglobinopathy card.
Approaches to the delivery of screening

There are two approaches to the delivery of the screening programme dependant on whether a Trust is defined as high prevalence or low prevalence.

‘High prevalence’ is defined as an area where the estimated fetal prevalence of sickle cell disease is 1.5 per 10,000 pregnancies or greater. ‘Low prevalence’ is where the estimated fetal prevalence of sickle cell disease is lower than this figure. The designation of high and low prevalence is kept under review based on newborn screening carrier results. The list of high and low prevalence Trusts can be found at sct.screening.nhs.uk/evaluationsreviews/.

In Trusts defined as covering high prevalence populations, laboratory sickle cell and thalassaemia screening should be offered to all women. The testing algorithm is given in Figure 3.

Areas defined as low prevalence will be required to offer screening for thalassaemia to all women using the routine blood indices. Further laboratory screening will be carried out on all women with defined abnormalities of red cell indices; those with high-risk family origins as determined by the family origin questionnaire; and those women who request screening. The testing algorithm is given in Figure 4. The family origin questionnaire is given in Appendix 1.

In all cases a failsafe system should be in place to ensure that all eligible women have been offered screening. Map of medicine pathways for the linked antenatal and newborn screening pathways are available at sct.screening.nhs.uk/carepathways. Two papers describing failsafe points across the antenatal and newborn screening pathways are available at sct.screening.nhs.uk/cms.php?folder=2421

Organisation of laboratory screening services

Screening is seen as part of routine antenatal care, provided by a locally-based service with adequate haematology expertise and experience. No minimum specimen throughput has been specified (unlike newborn screening), although where small numbers affect the timeliness of reporting, centralisation may be required. The introduction of pathology modernisation may have some impact. However it is essential that the NHS Sickle Cell & Thalassaemia Screening Programme algorithms are followed and standards are met, including requirements for data collection. Co-ordination of pathology services across chemistry and haematology can allow the sharing of HPLC equipment, for example with that used for measurement of Hb A1c, to allow optimal efficiency of the programme. If this is undertaken, it is essential that HbA2 is analysed by a buffer/column elution programme that clearly separates HbA2 from HbA. Some areas of the country have found it worthwhile combining the programme across several Trusts. Where this is the case, laboratories must have a risk-assessed protocol for all aspects of the process detailing: responsibility for coverage; transfer of selected specimens; receipt of results in a timely manner; appropriate reporting; and referral to comply with CPA standard E6. It must be agreed who will provide KPI data and data for annual returns.

Laboratories that detect few screen-positive cases, such as those in very low prevalence areas, will need to link with centres of expertise that can provide diagnostic support for presumed positive cases.

Family origin questionnaire (FOQ)

Screening and diagnostic uses of the family origin question:

The FOQ is used in high and low prevalence areas (Appendix 1). For low prevalence areas this is the basis of determining which women to test for the haemoglobin variants and helps in the interpretation of laboratory results. In high prevalence areas all women will be tested for the haemoglobin variants and the questionnaire helps with the interpretation of the laboratory results. The need for testing of the baby’s father in possible cases of α0 thalassaemia in both low and high prevalence areas will also be determined from the family origin questionnaire in conjunction with the red cell indices. All women should be screened for thalassaemia using the red cell MCH measurement.
The FOQ was developed from the initial literature review and research, funded by the programme centre, and further developed after an evaluation by Ethnos Research and Consultancy during the first phase of implementation of antenatal screening in low prevalence areas. The questions aim to determine family origins (ancestry) and are different to the census based self-assigned ethnic group questions. The current version of the questionnaire is given in Appendix 1. If the FOQ is incorporated into an electronic requesting system, the number of options available must not be fewer than those on the 3-part paper version. If any modifications of the FOQ are being considered, for instance as part of a combined antenatal screening request form, the programme centre should be contacted for advice.

Using the family origin questionnaire to help decide which women to screen:
In low prevalence areas, the family origin questionnaire should be used as the tool to identify women at high risk and thus determine those who require laboratory screening for haemoglobin variants. The woman should be offered testing if she or her baby’s father is in a high risk group.

Using the family origin questionnaire to help interpret laboratory results:
A second purpose for collecting information about family origin (ancestral origin) is to assist laboratories with the interpretation of laboratory screening results, particularly in the interpretation of results indicating possible α or β thalassaemia. For α thalassaemia, people originating from certain areas of the Far East and Eastern Mediterranean are at higher risk of α0 thalassaemia (which in the homozygous state results in hydrops fetalis). Where a request has been made for antenatal screening but an FOQ has not been supplied, it must be assumed that the woman is from a high risk area.

Family origin is also relevant in the interpretation of red blood cell indices and is essential for accurate DNA prenatal diagnosis to ensure that the relevant genotypes are included in the DNA analysis of fetal samples. Thus family origin is needed by laboratories even in areas where universal laboratory screening is applied.

Situations requiring particular care
Fertility treatment – donor gametes
If the pregnancy has been achieved by the use of a donor egg then the screening results on the woman will not be informative so the baby’s father should always be tested to ensure that this is not a high risk pregnancy. If donor sperm has been used then it may be appropriate to refer back to the fertility clinic if the screening results on the woman show that she is a carrier for a haemoglobinopathy.

Adoption
If either parent has been adopted, the FOQ information may not accurately reflect the true family origins. Such cases should be treated as high risk and have full laboratory screening.

Bone marrow transplants
In women who have received a bone marrow transplant, the haemoglobin results on her blood specimen will not necessarily indicate the genetic make up of the fetus. The baby’s father should always be tested to ensure that this is not a high risk pregnancy. Caution should be exercised in the interpretation of any haematology results in this instance. If DNA confirmation of mother’s status is required then pre-transplant DNA or DNA obtained from hair follicles should be used.
Table 5: Maternal conditions requiring testing of the baby’s father as part of the antenatal screening programme

(i) Significant maternal haemoglobinopathies
- these should be detected by antenatal screening and are important for maternal care

Hb SS
Hb SC
Hb SD\textsuperscript{Punjab}
Hb SE
Hb SO\textsuperscript{Arab}
Hb S/Lepore and Hb Lepore/\beta\ thalassaemia
Hb S/\beta\ thalassaemia
Hb S/\delta\beta\ thalassaemia
Hb H disease (--/-\alpha)
\beta\ thalassaemia major/intermedia
Hb E/\beta\ thalassaemia

(ii) Carrier states in mother

Hb AS
Hb AC
Hb AD\textsuperscript{Punjab}
Hb AE
Hb AO\textsuperscript{Arab}
Hb A Lepore
\beta\ thalassaemia carrier
\delta\beta\ thalassaemia carrier
\alpha^4\ thalassaemia carrier (--/\alpha\alpha)
HPFH

(iii) Any compound heterozygote state including one or more of the above conditions

(iv) Any homozygous state of the above conditions

Laboratory screening for haemoglobin variants

The following techniques can be used in first-line screening for haemoglobin variants:
- high performance liquid chromatography (HPLC) preferably with continuous gradient elution
- isoelectric focusing (IEF)
- capillary electrophoresis (CE)

Abnormal results should be confirmed by a different technique to the original and which is appropriate for the likely variant:

<table>
<thead>
<tr>
<th>Initial Method</th>
<th>Confirmatory Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>cellulose acetate electrophoresis (CAE)/IEF/CE</td>
</tr>
<tr>
<td>IEF</td>
<td>CAE/HPLC/CE</td>
</tr>
<tr>
<td>CE</td>
<td>acid agar electrophoresis/ HPLC/IEF</td>
</tr>
</tbody>
</table>

In most circumstances sickle solubility testing can be used as confirmation of an initial screen that suggests the presence of sickle haemoglobin but special care must be taken when it is known that Hb S co-elutes with another haemoglobin variant (such as Hb C) in the first line test. For further information see page 28 number 3. There is an inherent unreliability in the sickle solubility test with the risk of false positive and false negative results. For example, in cases of co-eluting haemoglobin variants S and C, a negative solubility test cannot be presumed to indicate haemoglobin C alone. In all cases where haemoglobins co-elute, it is necessary to use an additional confirmatory technique, for example cellulose acetate electrophoresis or iso-electric focusing.

Screening for thalassaemia:

Routine measurement of blood indices includes measurements of MCH and MCV. MCH should be used to screen for the risk of thalassaemia.
Other supporting investigations:

Hb A₂ measurement:
HPLC and CE methods are acceptable for the quantitation of Hb A₂. IEF and scanning densitometry are not acceptable.
No confirmatory test is necessary if the Hb A₂ is raised (but not greater than 8%) and the red cell indices are typical of a carrier of β thalassaemia.

Hb F measurement:
HPLC, CE or two minute alkali denaturation is acceptable.
The Kleihauer test is not appropriate for measurement but is useful to confirm the identity of Hb F.

General laboratory considerations
Users should be aware that the laboratory handbook highlights common analytical and diagnostic issues but every laboratory should follow the principles of good laboratory practice including satisfying themselves that they understand the capabilities and limitations of their chosen technique. The equipment and protocol chosen must fulfil the requirements of the screening programme and demonstrate suitable performance on EQA.
The use of rules to screen samples for further action/reporting or the use of post analytical data analysis algorithms are not recommended without software quality control procedures, including regular process audit to ensure that quality is not compromised. It is essential that the process is risk assessed and that there are failsafe mechanisms in place. Raw data including chromatograms must always be reviewed and any post analytical procedures (including algorithms) must be fully documented and traceable to ensure consistency of quality should staffing change over time.

If HPLC is used as the screening technique, it is essential to check and maintain the positions of the windows that are used as the first stage identification of any variants found. This is generally done by adjusting the column temperature or the flow rate so that the Hb A₂ peak appears at a standard time, but some analysers may use a different approach and the manufacturer’s guidelines should be followed. This is just as important as the calibration of the Hb A₂ and Hb F levels and should be checked on a daily basis. The column must be replaced once it has performed the recommended number of analyses as stated by the manufacturer.

For all analytical techniques, appropriate controls must be included wherever possible. The manufacturer’s published recommendations should be followed.

If IEF or electrophoresis is used, then control haemoglobins must be run with each plate.

It is often necessary to return to the original specimen bottle to check the identification details. For this reason it should be standard practice to ensure that any labels affixed when the specimen is received in the laboratory do not obscure the written identity or identity label already attached. The specimen bottle should also be checked at reception to ensure that it is within its expiry date.

Selecting an analytical system
When choosing an analytical system for use in antenatal screening for sickle cell and thalassaemia, some general considerations need to be borne in mind.

1 A national value for Hb A₂ of equal to or greater than 3.5% has been set as the action point in the diagnosis of carriers of β thalassaemia. There are two action points for Hb F. If the MCH is equal to or greater than 27pg, the action point for Hb F is greater than 10%. However, if the MCH is less than 27 pg, the action point is greater than 5.0%. The chosen system must 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 antenatal screening programme. Quantitation at different levels may be needed for other clinical purposes.

2 Analytical systems should be able to detect at least the most common Hb A₂ variant Hb A₂ \text{Prime} (A₂') and give an accurate quantitation of Hb A₂ plus A₂ \text{Prime}. If a small peak which could be an Hb A₂ variant elutes separately after the main Hb A₂ peak in a patient with an MCH below the cut off
point (<27 pg), further investigation will be required if the addition of these peaks brings the total Hb A₂ equal to or greater than 3.5%.

3 Step-wise gradients may produce co-elution of some haemoglobin variants. The most notable is the co-elution of haemoglobins S and C. Conversely such gradients may also split other haemoglobin variants into two peaks for example Hb D_punjab.

4 Peaks should be clearly separated when accurate quantitation is required. Sophisticated integration and the use of calibration factors cannot make up for poor chromatography.

5 Labs should understand how the integration takes place and be aware that peaks measured on sloping baselines or on shoulders of adjacent peaks are likely to be less reliable.

6 If using equipment or an elution programme for more than one analyte, (e.g. Hb A₂ and Hb A₁c) labs should ensure that the quantitation of Hb A₂ and Hb F is not compromised. This may require a different column/buffer system.

7 If capillary electrophoresis is used, appropriate control material must be used to ensure optimal analytical performance. OD levels greater than 0.07 and the presence of sufficient Hb A are required to ensure ‘zoning’ and enable the provisional identification of haemoglobins present in the sample. If failure to zone is due to low OD, this is usually related to the amount of haemoglobin in the sample. This should be corrected by increasing the haemoglobin to diluent ratio. Extreme care is needed if extraneous haemoglobin is added to a clinical sample to allow zoning. The addition of haemoglobins which were not present in the initial sample will make the chromatogram more difficult to interpret and may lead to misinterpretation of the results. Such modified samples should be analysed with a unique identifier distinguishable from both the original specimen and from any other clinical sample.
Problems with the measurement and interpretation of Hb A₂

A UK NEQAS project funded by the Sickle Cell and Thalassaemia screening programme has shown biases between different analysers for Hb A₂. The full report is available at sct.screening.nhs.uk/evaluationsreviews. The screening programme is working with the international bodies of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the International Council for Standardization in Haematology (ICSH) and the World Health Organisation (WHO), who have also recognised the problem and are working with manufacturers.

Haematology laboratories in the UK should be aware of these problems and ensure they have optimised their methods as far as possible. When considering replacement purchases, haematology laboratories should review all available evidence including the UK NEQAS report.

Besides the difficulties associated with the separation and quantitation 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, this is not a problem as long as the percentage of Hb A is greater than Hb S.

For analysers that separate and measure Hb A₂ in the presence of HbE, caution should be used when interpreting this value. Preliminary results suggest that existing reference ranges are not applicable.

In the presence of an alpha chain variant it can be difficult to obtain a reliable Hb A₂ value. For this reason, where the mother has an MCH of less than 27 pg and is found to have a suspected alpha chain variant or a haemoglobin variant that co-elutes or obscures the Hb A₂, the baby’s father should be offered screening (this is estimated to be about 150 fathers annually in England). DNA studies should be performed when the father has an MCH of less than 27 pg with a suspected alpha chain variant or a variant which co-elutes or obscures the Hb A₂ and the mother is known to have beta thalassaemia, or has Hb S or E or Lepore and/or is at risk of alpha zero thalassaemia.

Hb A₂ values of 4.0% and above with normal indices may indicate a carrier of β thalassaemia. In this case:-

- reanalyze FBC
- repeat Hb A₂ to confirm the value
- consider B12/folate deficiency or liver disease/alcohol or HIV infection. If the results remain the same then the baby’s father should be tested.

Hb A₂ values less than 4.0% with normal red cell indices and an Hb F level of less than or equal to 10% can usually be regarded as not significant for screening purposes.

Iron deficiency

The Hb A₂ level may be lowered by up to 0.5% in cases of severe iron deficiency anaemia, however screening for haemoglobin variants and thalassaemia should proceed without regard to iron deficiency, suspected or proven.

Any decrease in MCH should be regarded as potentially due to a haemoglobinopathy and the Hb A₂ should be measured. If the Hb A₂ is equal to or greater than 3.5% or if the woman’s haemoglobin is less than 80 g/L and the Hb A₂ is between 3.0% and 3.5%, testing of the baby’s father is recommended. It may be appropriate to simultaneously investigate pregnant women for iron deficiency, using ferritin or zinc protoporphyrin (ZPP) but this is not specifically part of the screening protocols.

In pregnant women there is no justification for delaying the investigation
of haemoglobinopathies whilst treating iron deficiency, as this will delay the process of identifying at-risk carrier couples who should be offered prenatal diagnosis.

Justification for above approach
Iron deficiency makes the red cell indices difficult to interpret and so an underlying haemoglobinopathy may be present. Severe iron deficiency anaemia (Hb < 80 g/L) can also reduce the Hb A₂ level slightly (up to 0.5%), but in practice this should not interfere with the screening protocol and testing of the baby's father should still be requested. If the father is found to be iron deficient, this could be a significant clinical finding and he should be referred to his General Practitioner.

Iron deficiency and α⁺ thalassaemia
Screening test results do not enable definitive differentiation of iron deficiency and α thalassaemia. Within the context of the screening programme, α⁺ thalassaemia is not regarded as a significant risk.

Screening for carriers of alpha zero thalassaemia (α₀ thalassaemia)

Methods of screening
The lack of a specific biomarker for the diagnosis of α thalassaemia carriers creates particular problems in the context of a screening programme. α⁺ Thalassaemia is not regarded as significant in the screening programme and policies are designed to detect only couples at risk of hydrops fetalis.

Policies have been designed to increase the positive predictive value of the screening algorithm and reduce false positives. In the past, high false positive rates resulted in a considerable workload for midwives and others responsible for requesting tests on the baby's father and further investigations, plus considerable anxiety for women and their families who were mostly carriers of α⁺ thalassaemia. This is neither a cost-effective use of resources nor a patient-centred approach.

Diagnosis
Molecular techniques should be reserved for the confirmation and diagnosis of α₀ thalassaemia when suspected in both parents. α Thalassaemia mutations may be both deletional and non-deletional, requiring the use of different diagnostic techniques. For the common deletional mutations many laboratories use GAP PCR based techniques and will therefore detect only those mutations included in their screening panels. Most do not screen routinely for rarer mutations.

Population estimates and ethnic distribution of α₀ thalassaemia
It is estimated that only a small number of cases of α thalassaemia major can be expected each year in England and Wales, with approximately half of these of Chinese family origin.

α₀ Thalassaemia is found most commonly in people of East Mediterranean (Cyprus, Greece, Sardinia or Turkey) and Southeast Asian origin (China, Hong Kong, Thailand, Taiwan, Cambodia, Laos, Vietnam, Burma, Singapore, Indonesia or Philippines). There are two common Mediterranean (--MED, --α(20.5)) and three common Southeast Asian deletional mutations (--SEA, --THAI, --FIL).

α₀ Thalassaemia has also been reported to occur at low frequencies in some Middle Eastern countries: the --MED allele in the UAE, Iran, Yemen, Kuwait, and Jordan; the --α(20.5) allele in Iran; and the --YEM allele in Yemenite families living in Israel. No α₀ thalassaemia alleles were found in reported studies of Egyptian and Saudi Arabian families. α₀ thalassaemia is rarely reported in patients of African, Pakistani and Indian origin. Only three Asian couples at risk of Hb Bart's hydrops fetalis are known in the UK at present. No African case of Hb Bart's hydrops fetalis has been reported. α₀ Thalassaemia is rarely observed in patients of British origin and no couple at risk of Hb Bart's hydrops fetalis has been reported. In these family origin groups the risk is small and in the context of screening, the cost benefit ratio is poor. Therefore the FOQ does not identify these groups as at risk for α₀ thalassaemia.
Further investigation of $\alpha^0$ thalassaemia

$\alpha$ Thalassaemia major is invariably fatal without treatment, resulting in hydrops fetalis due to severe fetal anaemia. If not detected, it can result in a stillbirth. A mother carrying a fetus with $\alpha$ thalassaemia major is at risk of obstetric complications such as toxaemia and hypertension, particularly in the third trimester of pregnancy. If a fetus with $\alpha$ thalassaemia major is transfused *in utero*, it is possible that it will survive and be born as a transfusion-dependent infant. Therefore if a pregnancy is considered to be at risk of $\alpha^0$ thalassaemia, a prenatal diagnosis should be considered to avoid the possibility of inadvertently transfusing an $\alpha$ thalassaemia major fetus *in utero*. Usually a baby only has Hb Bart's hydrops fetalis ($\alpha$ thalassaemia major) if both parents are carriers of $\alpha^0$ thalassaemia. Table 6 shows the $\alpha$ thalassaemia genotypes.

In cases where the fetus is at risk of Hb Bart's hydrops fetalis, ultrasound assessment at a Regional Fetal Medicine Centre can identify fetal hydrops and measure the fetal middle cerebral artery peak systolic velocities, which are increased in fetal anaemia. If the middle cerebral artery peak systolic velocities are normal, the fetus is not significantly anaemic and the couple can be reassured, avoiding invasive treatment and the associated risks.

Table 6: $\alpha$ Thalassaemia genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha/\alpha$</td>
<td>Normal</td>
</tr>
<tr>
<td>$-\alpha/\alpha$</td>
<td>Alpha plus ($\alpha^+$) thalassaemia (heterozygote)</td>
</tr>
<tr>
<td>$-\alpha/-\alpha$</td>
<td>Alpha plus ($\alpha^+$) thalassaemia (homozygote)</td>
</tr>
<tr>
<td>$-/-\alpha$</td>
<td>Alpha zero ($\alpha^0$) thalassaemia (heterozygote)</td>
</tr>
<tr>
<td>$--\alpha$</td>
<td>Haemoglobin H disease</td>
</tr>
<tr>
<td>$-/--$</td>
<td>Alpha thalassaemia major (homozygote)</td>
</tr>
<tr>
<td>$--/--$</td>
<td>Hb Bart’s hydrops fetalis</td>
</tr>
</tbody>
</table>

The recommended approach to screening and $\alpha^0$ thalassaemia

The screening programme is specifying the risks associated with this approach. It includes in its guidance, specific conditions unlikely to be detected and those family origins where there may be a low chance of an abnormal variant being detected.

Two sets of information from the pregnant woman are combined as the screen:

1. Is the MCH < 25 pg from the full blood count?
2. Is the woman's family origin (see Appendix 1 - Family Origin Questionnaire) from: China (including Hong Kong), Southeast Asia (especially Thailand, Taiwan, Cambodia, Laos, Vietnam, Burma, Malaysia, Singapore, Indonesia or Philippines), Cyprus, Greece, Sardinia, Turkey, or unknown?

If the answer to both questions is 'yes', testing of the baby's father should be requested if he is also from a high risk area.

If the baby's father is suspected to have $\alpha^0$ thalassaemia, the couple should be counselled and samples sent for DNA analysis for common $\alpha^0$ thalassaemia mutations.

If one parent is a suspected carrier of $\alpha^0$ thalassaemia and the other is a carrier of $\beta$ thalassaemia (and is also from one of the high risk groups for $\alpha^0$ thalassaemia with an MCH <25 pg), that parent should be screened for $\alpha^0$ thalassaemia by DNA analysis.

DNA analysis of samples and $\alpha^0$ thalassaemia

The policy guidance developed by the National Screening Programme should mean the DNA analysis is only done in a limited number of cases and not usually in cases where $\alpha^+$ thalassaemia is suspected.

Testing for the common $\alpha$ thalassaemia deletional mutations is usually carried out using multiplex GAP PCR. Users should be
aware that this technique is mutation specific and will only detect the mutations in the testing panel. Additionally, non-deletional mutations will not be detected using this approach. Southern blotting remains in use in some laboratories to facilitate the characterisation of copy number variations. Newer techniques such as multiple ligation-dependent probe amplification (MLPA), comparative genomic hybridisation array (CGH array) and next generation sequencing are also increasingly used to detect novel copy number variations and mutations.

Laboratories providing DNA testing services for thalassaemia should be CPA accredited. Those which are also part of the UK Genetic Testing Network (UKGTN) are listed on the UKGTN web site (ukgttn.nhs.uk).

Evidence for above approach

Published studies have shown that 99% of $\alpha^0$ thalassaemia cases have an MCH < 25 pg. Many have occasional red cells containing Hb H inclusions but these are not always detectable by routine screening. In a series of 270 carriers of $\alpha^0$ thalassaemia diagnosed by DNA analysis in UK, only two patients had an MCH between 25 and 26 pg – one with liver disease (quoted in BCSH Guidelines, 1998). Findings in a study from Sheffield\(^1\) which undertook DNA analysis in 425 pregnant women with an MCH < 27 pg showed that all cases of $\alpha^0$ thalassaemia had an MCH < 25 pg and would have been detected by using an ethnic question alone, which supports the screening programme’s approach.

\(^1\) ‘Is routine molecular screening for common a thalassaemia deletions necessary as part of an antenatal screening programme?’ Sorour Y, Heppinstall S, Porter N, Wilson GA, Goodeve AC, Rees D, Wright J. J Med Screen 2007;14:60-1
Figure 3 - Testing algorithm for laboratory screening in HIGH PREVALENCE areas (RF = Report Format)

- **FBC**
- **HPLC**

**Hb variant**
- If MCH <25pg and both parents are from high risk area** consider coexisting alpha0 thalassaemia
  - Hb S, Hb C, Hb D
  - Hb E, Hb Q^ variant
  - Hb Lepore
  - Test baby’s father (RF2 or 8)
  - Refer to Consultant Haematologist* (RF3a or 3b)

- Other variant
  - Hb A2 ≥3.5% beta thal carrier
  - Test baby’s father # (RF4a or 4b)
  - Consider family origin of both parents (from ROQ) **
    - High risk of alpha0 thalassaemia in mother**
    - Low risk of alpha0 thalassaemia in mother**
    - Test baby’s father if also from high risk area** (RF6a or 6b)
    - No further action (RF7a)

**MCH <27pg**
- Hb A2 <3.5%
  - Test baby’s father (RF5b)
  - Iron deficiency/ alpha thal
    - No further action (RF7b)

- Hb F >5%
  - Test baby’s father (RF4a or 4b)
  - No further action (RF1)

**MCH ≥27pg**
- Hb A2 ≤4.0% or Hb F ≤10%

- Hb A2 >4.0% or Hb F >10%
  - Test baby’s father # (RF4a or 4b)
  - Consider family origin of both parents (from ROQ) **
    - Low risk of alpha0 thalassaemia in mother**
    - No further action (RF7a)

- Hb F >5%
  - Test baby’s father (RF4b or 5a)
  - No further action (RF1)

- Hb A2 ≤4.0% and Hb F ≤10%
  - Test baby’s father (RF4b or 5a)
  - No further action (RF1)

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* Refer analytical results to consultant for an opinion on the need for a clinical referral or consult the laboratory support service helpline.

** Consider at high risk if any ethnic origins in China (including Hong Kong), Taiwan, Thailand, Cambodia, Laos, Vietnam, Indonesia, Burma, Malaysia, Singapore, Philippines, Cyprus, Greece, Sardinia, Turkey, or if ethnic/family origin is uncertain/unknown.

# In all cases consider coexisting α0 thalassaemia if both parents are from a high risk area and MCH <25pg.

Reconsider low risk couples if fetal anaemia/hydrops seen on ultrasound scanning or if family history of hydrops fetalis.
Risk assessment for antenatal screening in high prevalence areas

Because of the way that screening programmes are designed, particularly in the use of action values, it is inevitable that there will be some false positives as a result of screening. These are women who have a positive result from the screening test but when further tests have been performed they do not have one of the designated haemoglobins or thalassaemia.

Conditions likely to be missed using proposed algorithm assuming that the family origin questionnaire has been completed accurately:

- ‘Silent’ or ‘near silent’ β thalassaemia carriers. Some β thalassaemia carrier genotypes are associated with borderline Hb A2 levels, and an action value of 3.5% with an MCH < 27 pg will miss some cases. Examples of such mutations include the CAP+1 A>C, IVSI-6 T>C, -101 C>T and Poly A (A>G) or (T>C).
- Possibly some β thalassaemia carriers obscured by severe iron deficiency anaemia.
- β Thalassaemia carriers with a co-existing δ chain mutation which is silent with the first line screening technique, or who have co-existing δ thalassaemia.
- β Thalassaemia carriers with co-existing Hb H Disease, as some cases have normal Hb A2 values.
- α0 Thalassaemia occurring outside the defined at risk family origins (see page 31 for further information) or in those women with an MCH ≥ 25 pg.
- δβ Thalassaemia carriers with Hb F ≤ 5%.
- γδβ Thalassaemia carriers.
- Dominant haemoglobinopathies in the baby’s father when the woman is Hb AA, (but these are very rare and should be suggested by the family history).
- Any significant haemoglobin silent with the first line screening.
- Any significant haemoglobin masked by an unreported bone marrow transplant and/or adoption.
- Any significant haemoglobin present in donor egg or sperm where the donor is undeclared or untested.
Figure 4 - Testing algorithm for laboratory screening in LOW PREVALENCE areas (RF = Report Format)

- **FBC**
  - MCH ≥ 27pg
    - Consider family origin of both parents
      - Low risk family origin***
        - No further action (RF0)
      - High risk family origin***
        - HPLC
          - Hb A₂ ≥ 3.5%
            - beta thal carrier
          - Hb A₂ < 3.5%
            - MCH < 25pg
              - Test baby's father # (RF4a or 4b)
            - MCH ≥ 25pg
              - Iron deficiency/alpha*thal
                - Other variant
        - Hb variant
          - Hb S, Hb C, Hb D
          - Hb E, Hb O²Ab
          - Hb Lepore
            - Test baby's father (RF2 or 8)
            - Refer to Consultant Haematologist* (RF3a or 3b)
          - Other variant
            - Test baby's father (RF1)
            - No further action (RF4b or 5a)
          - No Hb variant
            - Hb A₂ ≤ 4%
              - Hb F ≤ 10%
            - Hb A₂ > 4.0%
              - or Hb F > 10%

  
  - MCH < 27pg
    - HPLC
      - Hb F > 5%
        - Hb variant
          - Hb S, Hb C, Hb D
          - Hb E, Hb O²Ab
          - Hb Lepore
          - Test baby's father # (RF5b)
          - Other variant
            - Test baby's father (RF5b)
            - No further action (RF7a)
            - Iron deficiency/alpha*thal
              - Other variant
      - MCH ≥ 25pg
        - Consider family origin of mother (from FOQ) **
          - High risk of alpha*thassaemia in mother**
            - Test baby's father if also from high risk area (RF6a or 6b)
          - Low risk of alpha*thassaemia in mother**
          - No further action (RF7b)
    
- Refer to Consultant Haematologist* # (RF3a or 3b)

* Refer analytical results to consultant for an opinion on the need for a clinical referral or consult the laboratory support service helpline.
** High risk if any ethnic/family origins in China (including Hong Kong), Taiwan, Thailand, Cambodia, Laos, Vietnam, Burma, Malaysia, Singapore, Indonesia, Philippines, Cyprus, Greece, Sardinia, Turkey, or if ethnic/family origin uncertain/unknown.
*** Low risk or high risk as determined by the family origin questionnaire. Note - If baby's father is in high risk ethnic group, test the mother's sample regardless of her family origins.
# In all cases consider coexisting α*thalassaemia if both parents are from a high risk area and MCH < 25pg.

Reconsider low risk couples if fetal anaemia/hydrops seen on ultrasound scanning or if family history of hydrops fetalis.
Risk assessment for antenatal screening in low prevalence areas

Because of the way that screening programmes are designed, particularly in the use of action values, it is inevitable that there will be some false positives as a result of screening. These are women who have a positive result from the screening test but when further tests have been performed they do not have one of the designated haemoglobins or thalassaemia.

Conditions likely to be missed using proposed algorithm assuming that the family origin questionnaire has been completed accurately:

- ‘Silent’ or ‘near silent’ \( \beta \) thalassaemia carriers. Some \( \beta \) thalassaemia carrier genotypes are associated with borderline Hb A\(_2\) levels, and an action value of 3.5% in conjunction with an MCH of <27pg will miss some cases. Examples of such mutations include the CAP+1 A>C, IVSI-6 T>C, -101 C>T and Poly A (A>G) or (T>C).
- Any \( \beta \) thalassaemia carriers where the MCH is ≥ 27pg.
- Possibly some \( \beta \) thalassaemia carriers obscured by severe iron deficiency anaemia.
- \( \beta \) Thalassaemia carriers with a co-existing \( \delta \) chain mutation which is silent with the first line screening technique, or who have co-existing \( \delta \) thalassaemia.
- \( \beta \) Thalassaemia carriers with co-existing Hb H Disease, as some cases have normal Hb A\(_2\) values.
- \( \alpha^0 \) Thalassaemia occurring outside the defined at risk family origins (see page 31 for further information) or in those women with an MCH ≥ 25pg.
- \( \delta\beta \) Thalassaemia carriers with Hb F ≤ 5%.
- \( \gamma\delta\beta \) Thalassaemia carriers.
- Dominant haemoglobinopathies in the baby’s father when the woman is Hb AA, (but these are very rare and should be suggested by the family history).
- Any significant haemoglobin silent with the first line screening technique.
- Hb S, C, D\(^{\text{Punjab}}\), E, O\(^{\text{Arab}}\) outside the defined ‘at risk’ family origins.
- Any significant haemoglobin masked by an unreported bone marrow transplant and/or adoption.
- Any significant haemoglobin present in donor egg or sperm where the donor is undeclared or untested.
Interpretation and reporting of antenatal screening results

These guidelines on the reporting of laboratory data and the clinical and genetic implications of that data are an attempt to standardise communication with professionals, patients and parents.

The guidelines should provide sufficient information to allow all normal, and over 95% of abnormal, reports 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 samples, or family studies, before a useful clinical diagnosis can be achieved.

It is important to realise that almost all haemoglobinopathy results are used to either diagnose, or exclude the diagnosis of, an inherited condition. The implication of this is that the data and conclusions are likely to be recorded and made use of for the remainder of that individual’s life.

If an individual has had a blood transfusion and any of the transfused red cells are still present, misleading data and conclusions may result. It is therefore essential that clinicians realise this fact and it may be prudent to have a universal footnote on all haemoglobinopathy results such as: ‘Results may be misleading if there has been a blood transfusion in the last four months’.

The small proportion of results that are not covered by the following guidelines may be due to the presence of an unusual variant or thalassaemia mutation, or to an interacting clinical situation such as recent blood transfusion, severe iron deficiency anaemia, liver disease or B12/folate deficiency. Haemoglobin values of less than 80 g/L should be treated with caution because, depending on the red cell indices, this may indicate iron deficiency, Hb H disease, haemolytic anaemia or β thalassaemia intermedia. These results will need discussion with a senior clinician or clinical scientist for appropriate reporting, and testing of the baby's father. In these circumstances if the Hb A₂ is between 3.0% and 3.5%, testing of the baby’s father is recommended, as severe iron deficiency may lower the Hb A₂ value.

It is not possible to separate all haemoglobin variants independently using screening methods. It must be recognised therefore that a second haemoglobin variant may be migrating/eluting with Hb A. In other cases, one clinical condition may be masking another.

### First line testing shows the presence of Hb A only.

1. If the first line tests only show a single major Hb peak (band) of Hb A and the MCH ≥ 27 pg and the Hb A₂ is normal (≤ 4.0%) and the Hb F is ≤ 10% and the Hb is ≥ 80 g/L

   There is negligible risk in the pregnancy associated with an abnormal haemoglobin or thalassaemia. **Use report format 1.**

2. If the results are as in 1 above but Hb A₂ > 4.0% but < 8.0%, there is a risk in the pregnancy associated with the presence of β thalassaemia. **Use report format 4b.** If the Hb A₂ > 8.0%, check for other variants.

3. If the results are as in 1 above but MCH < 27 pg and Hb A₂ ≥ 4.0% but < 8.0%, there is a risk in the pregnancy associated with the presence of β thalassaemia. **Use report format 4a.** If the results are as in 1 above but the MCH is between 25 and 27 pg and Hb A₂ ≥ 3.5% but < 4.0%, there is a possible risk in the pregnancy associated with the presence of β thalassaemia. **Use report format 4b.** If the Hb A₂ > 8.0% check for other variants particularly Hb Lepore.

4. If the results are as in 1 above but MCH < 27 pg and Hb A₂ < 3.5%, consider whether this is the measurement of the total Hb A₂. If the patient has a delta chain variant or an alpha chain variant, a second Hb A₂ peak/band will be present and must be included in the total Hb A₂ where appropriate. If total Hb A₂ (Hb A₂ plus Hb A₂ variant) ≥ 3.5% then there maybe a risk in the pregnancy associated with the presence of β thalassaemia. **Use report format 4a or 4b.**
If the results are as in 1 above but Hb F > 10%, there is a risk in the pregnancy associated with the presence of HPFH. Use report format 5a. See note below about HPFH and \( \beta \) thalassaemia.

If the results are as in 1 above but MCH < 27 pg and Hb F > 5.0%, there is a risk in the pregnancy associated with the presence of \( \delta \beta \) thalassaemia. Use report format 5b. See note below about HPFH and \( \beta \) thalassaemia.

*Carriers of deletional HPFH have a raised Hb F level of 20-30%. There are two types found — only in individuals of African origin — the HPFH1 deletion and the HPFH2 deletion (called the Ghanaian deletion) — and both are usually associated with normal red cell indices. It is important to differentiate deletional HPFH from \( \delta \beta \) thalassaemia. The carrier state for \( \delta \beta \) thalassaemia is associated with a reduced MCH and an Hb F level usually in the range of 5-15%. Nondeletional HPFH (the heterocellular form) usually results in a more modest increase of Hb F (1-10%) in adults and is found in many populations. It is usually associated with normal red cell indices.

If the results are as in 1 above but MCH ≥ 25 pg but < 27 pg and Hb A2 < 3.5% it may indicate possible iron deficiency or \( \alpha^+ \) thalassaemia. There is negligible risk in the pregnancy. Use report 7b.

If the results are as in 1 above but MCH < 25 pg and Hb A2 < 3.5% and the woman is from a high risk area for \( \alpha^0 \) thalassaemia, it may indicate possible iron deficiency, homozygous \( \alpha^+ \) thalassaemia, or heterozygous \( \alpha^0 \) thalassaemia. Report format 6a should be used stating that there is no evidence of an abnormal haemoglobin and recommending iron status is checked, but also stating that testing of the baby’s father is not required.

If the results are as in 1 above but MCH < 25 pg and Hb A2 < 3.5% and the woman is from a low risk area \( \alpha^0 \) thalassaemia, it may indicate possible iron deficiency, homozygous \( \alpha^+ \) thalassaemia, or heterozygous \( \alpha^0 \) thalassaemia. Report format 7a should be used stating that there is no evidence of an abnormal haemoglobin and recommending iron status is checked, but also stating that testing of the baby’s father is not required.

If the woman has confirmed Hb H disease, report format 6b should be used, offering testing of the baby’s father if he has family origins from one of the specified regions. This information is summarised in table 7.
Table 7: Where the first line test shows a single peak in the position of Hb A

<table>
<thead>
<tr>
<th>Family origin</th>
<th>MCH pg</th>
<th>Total A2%</th>
<th>F %</th>
<th>Conclusion</th>
<th>Rep format</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>≥27</td>
<td>≤4</td>
<td>≤10</td>
<td>No evidence of Hb variant or thalassaemia</td>
<td>1</td>
</tr>
<tr>
<td>Any</td>
<td>≥27</td>
<td>&gt;4 but &lt;8.0</td>
<td>≤10</td>
<td>Possible β thalassaemia carrier</td>
<td>4b</td>
</tr>
<tr>
<td>Any</td>
<td>≥27</td>
<td>≥8.0</td>
<td>≤10</td>
<td>Check for variant haemoglobin</td>
<td>-</td>
</tr>
<tr>
<td>Any</td>
<td>≥27</td>
<td>≤4</td>
<td>&gt;10</td>
<td>Hereditary persistence of fetal haemoglobin</td>
<td>5a</td>
</tr>
<tr>
<td>Any</td>
<td>&lt;27</td>
<td>≥3.5 but &lt;8.0</td>
<td>≤10</td>
<td>β Thalassaemia carrier</td>
<td>4a</td>
</tr>
<tr>
<td>Any</td>
<td>25.1 to 26.9</td>
<td>≥3.5 but &lt;4.0</td>
<td>≤10</td>
<td>Possible β thalassaemia carrier</td>
<td>4b</td>
</tr>
<tr>
<td>Any</td>
<td>&lt;27</td>
<td>&gt;8.0</td>
<td>≤10</td>
<td>Check for variant haemoglobin, particularly Hb Lepore</td>
<td>-</td>
</tr>
<tr>
<td>Any</td>
<td>&lt;27</td>
<td>&lt;3.5</td>
<td>≤10</td>
<td>Check for a second Hb A2 due to either an alpha or a delta chain variant</td>
<td>-</td>
</tr>
<tr>
<td>Any</td>
<td>&lt;27</td>
<td>&lt;3.5</td>
<td>&gt;5.0</td>
<td>δ/β Thalassaemia carrier</td>
<td>5b</td>
</tr>
<tr>
<td>Any</td>
<td>25.1 to 26.9</td>
<td>&lt;3.5</td>
<td>≤10</td>
<td>Possible iron deficiency or α thalassaemia</td>
<td>7b</td>
</tr>
<tr>
<td>Mother high risk for α0 thalassaemia</td>
<td>&lt;25</td>
<td>&lt;3.5</td>
<td>≤10</td>
<td>Possible α0 thalassaemia carrier and/or iron deficiency. Baby’s father required only if both parents are from high risk areas</td>
<td>6a</td>
</tr>
<tr>
<td>Mother low risk for α0 thalassaemia</td>
<td>&lt;25</td>
<td>&lt;3.5</td>
<td>≤10</td>
<td>Possible iron deficiency or homozygous α+ thalassaemia or heterozygous α0 thalassaemia. Baby’s father not required</td>
<td>7a</td>
</tr>
<tr>
<td>Any</td>
<td></td>
<td></td>
<td></td>
<td>If Hb H is present, baby's father is required if he is high risk family origins for α0 thalassaemia</td>
<td>6b</td>
</tr>
</tbody>
</table>
First test suggests Hb A and Hb S.

Confirmation required:

If Hb S > 20% - sickle solubility test (see note on page 26 about the need for caution when using the sickle solubility test for confirmatory purposes).

If Hb S < 20% - the sickle solubility test may not be positive when the Hb S is below 20%, but a sickling preparation should show sickled cells.

**Hb S between 40 and 50%** - Is the band/peak in the A position actually A? If Hb S > Hb A the possibility exists of Hb Sβ+ thalassaemia with an unusually high expression of Hb A. Unless this is the case, it does not alter the antenatal risk – which is only for Hb S.

**Hb S > 50%** - consider Hb Sβ+ thalassaemia.

Cases of Hb Sβ+ thalassaemia will usually have a reduced MCH. With some HPLC systems, a peak of HbS adducts elutes in the Hb A0 window and so a person with Hb SS may appear to have Hb Sβ+ thalassaemia. Electrophoresis on CAE at alkaline pH is likely to elucidate the true situation. In the case of Hb Sβ+ thalassaemia the antenatal risk is for both Hb S and beta thalassaemia.

**Hb S < 30% and MCH < 25 pg** - possible co-existing α thalassaemia or iron deficiency.

The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb S, but the risk of hydrops should not be overlooked.

There is a risk in the pregnancy associated with the presence of Hb S. **Use report format 2 or 8 as appropriate.**

**Confirmation required:** IEF or alkaline and acid electrophoresis. Consider checking film for target cells.

**Hb C between 40 and 50%** - Is the band/peak in the Hb A position actually Hb A? This does not however alter the antenatal risk – which is only for Hb C.

**Hb C > 50%** - consider Hb Cβ+ thalassaemia.

The red cell indices are helpful as MCH is usually below 27 pg. In the case of Hb Cβ+ thalassaemia the antenatal risk is for both Hb C and beta thalassaemia.

**Hb C < 35%** - possible co-existing α thalassaemia or iron deficiency.

The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb C, but the risk of hydrops should not be overlooked.

There is a risk in the pregnancy associated with the presence of Hb C. **Use report format 2 or 8 as appropriate.**

First test suggests Hb A and Hb D.

The position of Hb D will depend upon the system in use. Some systems underestimate the Hb A2 level in the presence of Hb DPunjab. This should not cause a diagnostic problem except in a possible case of Hb D/β0 thalassaemia or Hb DD when the red cell indices are of more value.

Confirmation required: either IEF and acid electrophoresis or alkaline electrophoresis (e.g. cellulose acetate) and acid electrophoresis. There are several D haemoglobins, but the screening programme aims to detect Hb DPunjab. When Hb D is detected and it cannot be differentiated, testing of the baby’s father will identify the at risk couples. The Hb D will need to be confirmed as Hb DPunjab if the baby’s father is found to have Hb S. This will also apply if the mother has Hb S and the baby’s father is found to have Hb D.

**Hb D between 40 and 50%** - Is the band/peak in the Hb A position actually Hb A? This does not however alter the antenatal risk – which is only for Hb D.

**First test suggests Hb A and Hb C.**

The position of Hb C relative to other haemoglobins can vary considerably depending upon the system used. On some HPLC systems, Hb C gives a small additional peak of Hb C adducts in the S window. This is part of the Hb C fraction.
Hb D > 50% - consider Hb Dβ+ thalassaemia.

The red cell indices are helpful as MCH is usually below 27 pg. In the case of Hb Dβ+ thalassaemia the antenatal risk is for both Hb D and beta thalassaemia.

Hb D < 30% and MCH < 25 pg - possible co-existing α thalassaemia or iron deficiency. The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb D, but the risk of hydrops should not be overlooked.

There is a risk in the pregnancy associated with the presence of Hb D. Use report format 2 or 8 as appropriate.

First test suggests Hb A and Hb E.

By most HPLC methods, Hb E appears as a peak in Hb A₂ window and therefore the levels of both Hb E and Hb A₂ cannot be estimated accurately. By IEF there is a band in Hb E position and by alkaline electrophoresis (e.g. cellulose acetate), a band in the Hb C position. CE separates Hb E from Hb A₂.

Confirmation required: either IEF and acid electrophoresis, or alkaline electrophoresis (e.g. cellulose acetate) and acid electrophoresis.

Hb E between 35 and 50% - Is the band/peak in the Hb A position actually Hb A?

This does not however alter the antenatal risk – which is only for Hb E.

Hb E > 50% - consider Hb Eβ+ thalassaemia.

The red cell indices are unhelpful as the MCH is usually below 27 pg in patients who are carriers of Hb E, but most Hb Eβ+ thalassaemia patients will also have a reduced haemoglobin level and a Hb F > 4%.

In the case of Hb Eβ+ thalassaemia the antenatal risk is for both Hb E and beta thalassaemia.

Hb E < 25% and MCH < 25 pg - possible co-existing α thalassaemia or iron deficiency.

The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb E but this Hb is found predominately in those groups at highest risk for α0 thalassaemia so this should always be considered.

There is a risk in the pregnancy associated with the presence of Hb E. Use report format 2 or 8 as appropriate.

Hb OArab appears in different positions on different analysers but frequently appears as a peak just before Hb C. By IEF there is a band in Hb E position and by alkaline electrophoresis (e.g. cellulose acetate), a band in the Hb C position. CE separates Hb E from Hb A₂.

Confirmation required: either IEF and acid electrophoresis, or alkaline electrophoresis (e.g. cellulose acetate) and acid electrophoresis.

Hb OArab between 40 and 50% - Is the band/peak in the Hb A position actually Hb A?

This does not however alter the antenatal risk – which is only for Hb OArab.

Hb OArab > 50% - consider Hb OArab/β+ thalassaemia.

The red cell indices are helpful as MCH is usually below 27 pg. In the case of Hb OArab/β+ thalassaemia the antenatal risk is for both Hb OArab and beta thalassaemia.

Hb OArab < 30% and MCH < 25 pg - possible co-existing α thalassaemia or iron deficiency.

The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb OArab, but the risk of hydrops should not be overlooked.

There is a risk in the pregnancy associated with the presence of Hb OArab. Use report format 2 or 8 as appropriate.
First test suggests Hb A and Hb Lepore

Hb Lepore appears in different positions depending on the system used. Quite often it appears as an oddly shaped elevated Hb A2 peak with values up to 15% and therefore the levels of neither Hb Lepore nor Hb A2 can be estimated accurately. By IEF there is a band in Hb GPhiladelphia position and by alkaline electrophoresis (e.g. cellulose acetate), a band just faster than Hb S, but this may be difficult to separate from Hb S. The MCH is usually < 27 pg.

Confirmation required: either IEF and acid electrophoresis or alkaline electrophoresis (e.g. cellulose acetate) and acid electrophoresis.

The α thalassaemia risk needs to be considered in the light of the family origin of the patient – see algorithm for α thalassaemia. The major risk is for Hb Lepore, but the risk of hydrops should not be overlooked.

There is a risk in the pregnancy associated with the presence of Hb Lepore. Use report format 2.

Homozygotes and compound heterozygotes

Where a single major peak (band) is detected, this is most likely due to homozygosity for the Hb variant. However, co-existing thalassaemia (β and/or α) or the presence of more than one variant must not be overlooked. The red cell indices and Hb A2 level need to be taken into consideration. This may be particularly difficult in the case of EE/EBβ0 with or without α thalassaemia.

The risk in these pregnancies is associated with the haemoglobin variant detected and possible co-existing thalassaemia. Use report format 8.

General notes on reporting antenatal screening results

1 The sample date must be given as this can be essential if a person has had a recent blood transfusion.

2 Analytical fact must be separated from interpretative opinion. The factual results should be given and then a clear conclusion, which may include recommendations. If there is likely to be a delay in producing a final result which breaches the 3 day standard for reporting, an interim report, sufficient for the woman’s clinical care and for recommending the baby’s father testing, if necessary, should be issued.

3 The blood count should always be reviewed as it may be the only indication of α thalassaemia or anaemia. 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 RBC, MCH, MCV) must be included in the haemoglobinopathy report.

4 Since 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 (Hb AS) or Homozygous Sickle Cell (Hb SS). The convention recommended is for the haemoglobins present to be reported in the order of greatest to least percentage.

5 If no further action is required it is helpful to say so or to state that testing of the baby’s father is not required.

6 Where decisions are based on information derived from the FOQ, it is best practice to include that information on the report.

7 In conclusion, ensure that both the analytical facts and the conclusion message are clear, and that the report will lead to the action that is considered necessary and will not lead to inappropriate worry.

Recommended report formats

Laboratory reporting can be simplified by considering the conditions that are likely to be encountered in the antenatal screening programme. These will comprise:-

- those with no evidence of a haemoglobin variant or thalassaemia
- carriers of a haemoglobin variant (including sickle haemoglobin)
• thalassaemia carriers
• homozygote and compound heterozygote conditions of sickle haemoglobin and either another haemoglobin variant or thalassaemia.

Appendix 3 and appendix 4 give details of the antenatal screening ‘status codes’ and ‘outcome codes’ that are to be used for linking laboratory reports to maternity unit information systems and other computerised record keeping systems. It is possible that the comment about testing the baby’s father will not be needed in all laboratories if alternative protocols are used by the screening service to initiate such requests.

Report format 0 - for specimens screened by red cell indices only (Low prevalence area)
Report the red cell indices with the comments:-
1 No evidence of thalassaemia
2 Not tested for haemoglobin variants as mother and baby’s father from low risk group on family origin questionnaire
3 Testing of baby’s father not required

Report format 1 - for normals
Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:-
1 No evidence of an abnormal haemoglobin or thalassaemia
2 Testing of baby’s father not required

Report format 2 - for haemoglobin variant carriers on the specified list i.e. Hb S, Hb C, Hb D, Hb E, Hb OArab and Hb Lepore
Report the red cell indices, sickle cell solubility test (if performed) and the other chromatographic or electrophoretic results together with the comments:-
1 Hb ‘V’ carrier (Hb AV) - where V is the variant haemoglobin detected
2 No evidence of thalassaemia (remove this if the variant is Lepore or co-existing αthalassaemia is suspected)
3 Testing of baby’s father recommended

Consider discussion with consultant haematologist

Report format 3a - haemoglobin variant carriers where baby’s father testing is not required
Report the red cell indices, sickle cell solubility test (if performed) and the other chromatographic or electrophoresis results together with the comments:-
1 Hb ‘V’ carrier (Hb AV) of no known clinical significance - where V is the variant haemoglobin detected
2 No evidence of thalassaemia
3 Testing of baby’s father not required

In the case where the variant is Hb S the term ‘sickle cell carrier’ is preferred.

Report format 3b - haemoglobin variant carriers where baby’s father testing is required
Report the red cell indices, sickle cell solubility test (if performed) and the other chromatographic or electrophoresis results together with the comments:-
1 Hb ‘V’ carrier (Hb AV) of known clinical significance - where V is the variant haemoglobin detected or state if the variant is unknown
2 No evidence of thalassaemia (remove this comment if co-existing αthalassaemia is suspected)
3 Testing of baby’s father recommended

Consider discussion with consultant haematologist

Report format 4a - for βthalassaemia carriers
Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:-
1 Carrier of βthalassaemia
2 No evidence of sickle haemoglobin
3 Testing of baby’s father recommended
Report format 4b - for possible β thalassaemia carriers

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Possible carrier of β thalassaemia
2. No evidence of sickle haemoglobin
3. Testing of baby’s father recommended

Report format 5a – for HPFH

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Carrier of Hereditary Persistence of Fetal Haemoglobin
2. No evidence of sickle haemoglobin
3. Testing of baby’s father recommended

Report format 5b – for δβ thalassaemia trait

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Carrier of δβ thalassaemia
2. No evidence of sickle haemoglobin
3. Testing of baby’s father recommended

Report format 6a - for possible α thalassaemia carriers when both the mother and the baby's father are of high risk family origins

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Possible α thalassaemia carrier and/or iron deficiency
2. No evidence of sickle haemoglobin
3. Iron status should be checked but testing of the baby’s father should not be delayed
4. Testing of baby’s father recommended

Report format 6b - for confirmed Hb H disease

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Hb H disease
2. No evidence of sickle haemoglobin
3. Recommend referral to a Consultant Haematologist if not already being followed up
4. Testing of baby’s father recommended if he is of high risk family origins for α0 thalassaemia

Report format 7a – for possible α thalassaemia carriers when either parent is of low risk α0 thalassaemia family origins (MCH < 25 pg)

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Possible α thalassaemia carrier and/or iron deficiency
2. No evidence of sickle haemoglobin
3. Testing of baby’s father not required as one or both parents are of low risk family origins for α0 thalassaemia
4. Iron status should be checked but therapy not commenced unless iron deficiency is proven

Report format 7b – for possible α thalassaemia carriers (MCH 25 to 27 pg)

Report the red cell indices and the other chromatographic or electrophoretic results together with the comments:

1. Possible iron deficiency and/or α thalassaemia carrier
2. No evidence of sickle haemoglobin
3. Testing of baby’s father not required
4. Iron status should be checked but therapy not commenced unless iron deficiency is proven
Minimum criteria (standards) for laboratories undertaking antenatal screening

The non-cancer screening programmes are developing a quality assurance framework that will operate across the antenatal and newborn programmes. This framework covers the screening pathway from offer of testing through to entry into care, as well as the user experience, equity, governance and commissioning.

There are also programme specific quality assurance processes. The quality improvement minimum and achievable standards are given in detail in the NHS Sickle cell and Thalassaemia Screening Programme: Standards for the linked antenatal and newborn screening programme (sct.screening.nhs.uk/cms.php?folder=2493). These are referred to in the text as standard references.

1. The laboratory must be appropriately accredited with a nationally approved accreditation scheme such as Clinical Pathology Accreditation UK (Ltd), now formally part of the United Kingdom Accreditation Service (UKAS).
2. There must be a senior member of the laboratory staff at medical consultant or clinical scientist/biomedical scientist consultant level with expertise in haemoglobinopathy diagnosis, to be responsible for the haemoglobinopathy screening service, with defined lines of accountability for all laboratory aspects of the service.
3. The laboratory must adopt the testing algorithm, defined by the national screening programme, to determine those pregnancies at risk of sickle cell disease or thalassaemia. This testing algorithm sets out the conditions to be tested for and the analytical methods that can be used.
4. The acceptable sensitivity of the screening test (standard reference AO2a):
   - 95% sensitivity of FOQ to identify groups at risk of Hb variants (low prevalence areas)
   - 95% sensitivity of laboratory tests to identify carriers of thalassaemia and Hb variants (both high and low prevalence areas).
5. The laboratory must adopt the guidelines for the standardised reporting of antenatal screening results as defined by the national screening programme. These guidelines set out the wording to be used on laboratory reports in response to defined analytical data.
6. The laboratory must have a standard operating procedure for the antenatal sickle cell and thalassaemia screening service, describing the process of laboratory testing from initial receipt of the specimen until dispatching of the report.
7. There must be a documented risk management policy for the laboratory aspects of the antenatal sickle cell and thalassaemia screening service. This should include a risk assessment which describes the steps in the testing protocol where mistakes could occur and the procedures that have been implemented to minimise the risk of the mistake occurring. Where appropriate this should include a policy for any samples sent away for further analysis. It should also include documented procedures for the management and reporting of incidents.
8. The laboratory must participate in an accredited External Quality Assessment Scheme (EQAS), appropriate for antenatal sickle cell and thalassaemia (screening e.g. UK NEQAS), and must be able to demonstrate satisfactory performance as...
defined by the criteria specified by the EQA scheme organisers. It is expected that laboratories will participate specifically in UK NEQAS and will agree to share performance information with the Programme Centre.

9 A report or interim report must be provided within 3 working days of receipt of a specimen by the laboratory (standard reference AO2a).

10 The laboratory must participate in audit at local and regional level, including providing an annual data return to the Programme Centre to ensure the laboratory is meeting the Programme’s aims and objectives and to enable the production of an annual report (standard reference AO2a). This will request data on the number of various screening tests performed as well as the number of screen positives and negatives in the various categories. Detailed guidance has been developed to assist in the completion of these data returns. Data returns and guidance are available at sct.screening.nhs.uk/guidance. Data will also need to be collated and submitted in relation to the Key Performance Indicator (KPI) returns for the antenatal screening programme. This includes the number of samples accompanied by a completed FOQ (standard reference AO1aiii).

11 Screening laboratories must have fail-safe arrangements in place. This includes checking all samples have been received and all results are received and acted upon (positive and negative) in a timely manner, including links made to previous screening results All screen positive results must be reviewed regularly (standard reference AS1).

12 There must be regular meetings held at least quarterly to review accountability and responsibility and screen positive results across the linked programme, including fail-safe (standard reference LS1 and standard reference AS1 - acceptable standard 80% of meetings to be held at least quarterly).

13 The laboratory must be willing to release information on screening performance to any appropriate monitoring group of the National Screening Committee and the NHS Sickle Cell and Thalassaemia Screening Programme Centre, and be open to peer review visits and inspection by the commissioners or their representatives at any reasonable time, by mutual agreement.

14 Where some or all samples are referred to other laboratories there must be a risk-assessed protocol for all aspects of the process, detailing responsibility for coverage; transfer of selected specimens; receipt of results in a timely manner; appropriate reporting and referral to comply with CPA standard E6. It must be agreed who will provide KPI data and data for annual returns.

15 When reports received from third parties are transcribed into internal laboratory information systems, a full and exact copy of the report must be made. It is not acceptable to summarise or leave out information. The transcribed report should always be checked by an appropriate second person to ensure accuracy of transcription. If laboratories have a system to scan a copy into the laboratory information system this would be a preferred alternative. A report or an exact copy/photocopy of the report should also be included in the patient’s notes and included when referrals are made for counselling or advice.
Referral of antenatal samples to the DNA laboratories for haemoglobinopathy mutation analysis

The majority of couples at risk of having a child affected with β thalassaemia or sickle cell disease are identified initially by laboratory haematological techniques through the antenatal screening programme. The diagnosis of α thalassaemia is more complicated because DNA analysis is the only accurate way to distinguish between the two classes of α thalassaemia – alpha plus, i.e. α⁺ (-α/-α and α⁺/α⁺) and alpha zero, i.e. α₀ (–/α₀) thalassaemia. Due to high frequency of alpha plus thalassaemia in some populations and the low cost benefit ratio in a screening programme, DNA confirmation is not recommended for all possible cases of α thalassaemia. The screening programme relies on a strategy for diagnosis of α thalassaemia by combining haematological results and details of family origin as described.

It must be emphasised that although the antenatal screening programme guidelines are designed to identify most carriers for sickle cell disease, thalassaemia and related diseases, the screening protocol will not identify every couple at risk for every haemoglobinopathy. For example they are not designed to pick up couples at risk for Hb H disease or the extremely rare cases of couples from low risk groups on the FOQ who are at risk for Hb Bart’s hydrops fetalis syndrome.

Patient Information

Information materials on haemoglobin disorders for counsellors can be downloaded from the web site sct.screening.nhs.uk. Carrier leaflets are available on the website. The site contains information for health professionals, information for carriers and information for couples where one or both parents are carriers, including genetic risks of abnormal globin gene combinations and information about prenatal diagnosis for serious inherited conditions. A leaflet for couples on antenatal screening for haemoglobin disorders can be found at www.screening.nhs.uk/annbpublications

Testing of the baby’s father

This is done by the same haematological testing strategy as for maternal phenotype testing. If the baby’s father has a haemoglobinopathy that can interact with the maternal phenotype as depicted in Table 8, then the couple should be counselled and, if the parents so choose, fresh blood samples on both parents sent to a DNA referral laboratory with appropriate consent for molecular analysis, in preparation for prenatal diagnosis according to the following guidelines. A list of laboratories performing DNA analysis for prenatal diagnosis (PND) and pre-implantation genetic diagnosis (PGD) is given in Appendix 10. More information is available in the PND guidelines: sct.screening.nhs.uk/getdata.php?id=11962.

If the baby’s father is unavailable for testing or his haemoglobinopathy status is unknown, then a risk assessment should be done. The Programme supports the woman being offered prenatal diagnosis in this situation if she requests it. Prenatal diagnosis for some genotypes of sickle cell disease can be undertaken without the DNA of the baby’s father. Similarly, prenatal diagnosis for β thalassaemia can be undertaken without the DNA of the baby’s father, although the diagnosis will not be able to be given with such a high degree of certainty as when the baby’s father’s mutation is known.

Guidelines on the referral of blood samples for DNA Analysis

Blood samples will not be analysed without appropriate patient consent. The blood samples must be accompanied with a DNA laboratory referral form where there is confirmation that the patient has given appropriate consent for DNA to be analysed for the patient’s benefit, stored for possible further diagnostic tests for the benefit of the patient and, if they consent, for it to be used anonymously for controls in other diagnostic tests. The laboratory should be notified prior to sending samples.
Haemoglobin variants

1 If one parent carries Hb S and:
   • the other carries Hb S
     Blood samples are not required immediately on identification of the carrier to confirm the presence of Hb S by DNA analysis. If prenatal diagnosis (PND) has been chosen, fresh maternal and paternal blood samples should be sent at the same time as the fetal sample is referred.
   • the other carries Hb C
     Blood samples should not be sent to confirm Hb C by DNA analysis. The same guidelines apply as for Hb S above.
   • the other is thought to carry Hb O Arab, Hb D Punjab, Hb Lepore or a type of β thalassaemia trait
     If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples should be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

2 If one parent carries Hb S with α thalassaemia:
   Blood samples should not be sent to confirm and identify α thalassaemia, unless there is a real risk that it is the α0 thalassaemia type (only in cases where both parents are of high risk family origins for α0 thalassaemia). The same guidelines for referral apply as for Hb S above, for any combination of carrier status in the parents. There is no clinical interaction of Hb S with α thalassaemia and the genetic risks are the same as for Hb S without α thalassaemia. The type of α thalassaemia found in Hb S patients of Afro-Caribbean origin is almost always the α+ thalassaemia type, which poses no serious genetic risk to the fetus.

3 If one parent carries Hb E and:
   • the other carries β thalassaemia
     If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples should be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.
   • the other carries δβ thalassaemia or Hb Lepore
     If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.
   • the other carries α0 thalassaemia
     There is also the possibility of a hidden risk for α0 thalassaemia, as this can be masked in individuals who have family origins with a high risk for α0 thalassaemia who carry Hb E or β thalassaemia.
   • the other carries δβ thalassaemia or Hb Lepore
     If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.
   • the other carries α0 thalassaemia
     There is a possibility of a hidden risk for homozygous α0 thalassaemia, as the carrier state for α0 thalassaemia can be masked in individuals carrying Hb E and who have...
family origins with a high risk for $\alpha^0$ thalassaemia. If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples should be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

4 If one parent carries Hb O\textsubscript{Arab} and:
   - the other carries $\beta$ thalassaemia

If PND is being considered it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations and stratify the genetic risk prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

5 If one parent carries Hb Lepore and the other carries $\beta$ thalassaemia, Hb Lepore, Hb S, Hb E or Hb O\textsubscript{Arab}

If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

$\beta$ Thalassaemias

**When both parents are $\beta$ thalassaemia carriers**

If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

Note: In people of high risk family origins, the carrier state for $\alpha^0$ thalassaemia may also be present, as this can be masked by the carrier state for $\beta$ thalassaemia. It is important to determine the alpha genotype by DNA analysis in such couples.

When one parent carries $\beta$ thalassaemia and the other carries Hb Lepore, Hb S, Hb E or Hb O\textsubscript{Arab}

If PND is required it is highly recommended, if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

When one parent carries $\beta$ thalassaemia and the other parent carries probable or definite $\alpha^0$ thalassaemia

Fresh maternal and paternal blood samples should be referred for $\alpha^0$ thalassaemia mutation analysis if both parents are of high risk family origins. The couple may be at risk for homozygous $\alpha^0$ thalassaemia as the carrier state for $\beta$ thalassaemia can mask the co-inheritance of $\alpha^0$ thalassaemia.

When one parent is suspected of carrying $\delta\beta$ thalassaemia and the other carries $\beta$ thalassaemia, Hb Lepore or Hb O\textsubscript{Arab}

If PND is required it is highly recommended if time permits, that both maternal and paternal blood samples be referred with appropriate consent to confirm the mutations prior to fetal sampling. A fresh maternal sample will also be required to accompany the fetal sample when it is referred.

$\alpha$ Thalassaemias

**Alpha plus thalassaemia ($\alpha^+ \text{ thalassaemia or } -\alpha/-\alpha \text{ and } -\alpha/\alpha\alpha$)**

This is found in all ethnic groups, with a high (10-30%) carrier frequency in some parts of Africa, in Afro-Caribbeans and in South and Southeast Asia. Even if both parents are carriers, there is no risk to the fetus. Homozygous $\alpha^+$ thalassaemia ($-\alpha/-\alpha$) is not a clinically significant disorder with respect to genetic or obstetric complications, but can cause diagnostic confusion with carriers of $\alpha^0$ thalassaemia or iron deficiency.

- Heterozygotes (carriers, i.e. $-\alpha/\alpha\alpha$)
generally have a MCH of 25-28 pg and a normal Hb A2 level. Approximately one third of cases will be silent.

- Homozygotes (-α/-α) generally have a MCH below 25 pg and some have Hb H inclusions, the same as carriers for α0 thalassaemia (--/αα).

Affected individuals may be heterozygous for an alpha gene deletion (carrier genotype: -α/αα) or a rarer point mutation in one gene affecting gene expression (carrier genotype: αTα/αα), commonly called a non-deletional mutation. There are two common deletions (-α3.7 and -α4.2) and a number of less common non-deletional mutations. Usually, only the common deletions are tested for routinely.

### Alpha zero thalassaemia (α0 thalassaemia (--/αα))

This carries the potential for a clinically significant disorder. If both parents are carriers of α0 thalassaemia (--/αα), the couple is at a 1 in 4 risk of having a fetus with Hb Bart’s hydrops fetalis syndrome (--/-) and the mother runs the risk of obstetric complications, particularly in the third trimester of pregnancy. The mutations leading to Hb Bart’s hydrops fetalis are almost always gene deletions.

If one parent carries α0 thalassaemia (--/αα) and the other α+ thalassaemia (either -α/αα or -α/-α), then the couple is at risk of having a child with Hb H disease (--/-α). Prenatal diagnosis is not usually indicated for Hb H disease unless there are known higher risk mutations such as Hb Constant Spring.

### Summary of Guidance Notes

Table 8 summarises the main genetic risk combinations that require antenatal screening actions, according to the antenatal screening recommendations, and shows which cases require referral of samples for further studies by DNA analysis. For other haemoglobinopathy combinations, refer results for a consultant expert opinion.

### Minimum laboratory criteria (standards) for the DNA referral laboratories

The non-cancer screening programmes are developing a quality assurance framework that will operate across the antenatal and newborn programmes. This framework covers the screening pathway from offer of testing through to entry into care, as well as the user experience, equity, governance and commissioning.

There are also programme specific quality assurance processes. The quality improvement minimum and achievable standards are given in detail in the NHS Sickle cell and Thalassaemia Screening Programme: Standards for the linked antenatal and newborn screening programme (sct.screening.nhs.uk/cms.php?folder=2493). These are referred to in the text as standard references.

1. If the laboratory is performing PND or PGD it must be a member of the UK Genetic Testing Network (UK GTN) and comply with the quality criteria laid down by the UK GTN Steering Group.

2. The laboratory must be appropriately accredited with a nationally approved accreditation scheme such as Clinical Pathology Accreditation UK (Ltd), now formally part of the United Kingdom Accreditation Service (UKAS).

3. There must be a senior member of the laboratory staff at medical consultant or clinical scientist/biomedical scientist consultant level responsible for the DNA analytical service, with defined lines of accountability for all laboratory aspects of the service.

4. The acceptable standards for sensitivity of the diagnostic test (standard reference AO2b)
   - 99% sensitivity and specificity for Hb SS and SC at risk or affected pregnancies
   - 99% sensitivity and specificity for beta thalassaemia major at risk or affected pregnancies.

5. The acceptable standard for reporting is to have results available so that 90% of women can informed and counselled...
<table>
<thead>
<tr>
<th>Maternal carrier state</th>
<th>Paternal carrier state</th>
<th>Further studies by DNA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abnormalities detected</td>
<td>Testing of baby's father not required</td>
<td>None required</td>
</tr>
<tr>
<td>Any abnormal Hb/ thalassaemia</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&lt;sub&gt;Arab&lt;/sub&gt;, D&lt;sub&gt;Punjab&lt;/sub&gt;, Lepore, β thalassaemia</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb S</td>
<td>HPFH</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb S + α thalassaemia</td>
<td>As for Hb S</td>
<td>As for Hb S</td>
</tr>
<tr>
<td>Hb C</td>
<td>Hb S</td>
<td>None required until PND</td>
</tr>
<tr>
<td>Hb D</td>
<td>Hb S</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb O&lt;sub&gt;Arab&lt;/sub&gt;</td>
<td>Hb S, β thalassaemia</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb Lepore</td>
<td>Hb S, E, O&lt;sub&gt;Arab&lt;/sub&gt;, Lepore, β thalassaemia</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>Hb E</td>
<td>β thalassaemia, Hb Lepore, α thalassaemia (MCH &lt; 25 pg)</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>β thalassaemia</td>
<td>Hb S, E, O&lt;sub&gt;Arab&lt;/sub&gt;, Lepore, β thalassaemia</td>
<td>If PND required send bloods for mutation confirmation</td>
</tr>
<tr>
<td>β thalassaemia</td>
<td>α thalassaemia (MCH &lt; 25 pg)</td>
<td>Send bloods for mutation confirmation if high risk family origins for α&lt;sup&gt;0&lt;/sup&gt; thalassaemia</td>
</tr>
<tr>
<td>α thalassaemia (MCH of 25-27pg)</td>
<td>Testing of baby's father not required</td>
<td>None required</td>
</tr>
<tr>
<td>α thalassaemia (MCH &lt;25 pg)</td>
<td>Testing of baby's father not required</td>
<td>None required</td>
</tr>
<tr>
<td>1) Low risk α&lt;sup&gt;0&lt;/sup&gt; thal family origins</td>
<td>Test baby's father and if MCH &lt;25 pg irrespective of any other phenotype detected</td>
<td>Send maternal and paternal bloods for mutation confirmation</td>
</tr>
<tr>
<td>2) High risk α&lt;sup&gt;0&lt;/sup&gt; thal family origins or unknown</td>
<td>Test baby's father and if MCH ≥25 pg</td>
<td>None required</td>
</tr>
</tbody>
</table>
6 The laboratory must agree to collect a minimum dataset of information for monitoring purposes, as well as appropriate patient information for a computerised national database associated with sickle cell disease and thalassaemias.

7 The laboratory must have a standard operating procedure for the DNA work associated with the sickle cell and thalassaemia screening programme, describing the process of laboratory testing from initial receipt of the specimen until dispatching of the report.

8 There must be a documented risk management policy for the laboratory aspects of the DNA service. This should include a risk assessment that describe the steps in the testing protocol where mistakes could occur and the procedures that have been implemented to minimise the risk of the mistake occurring. Where appropriate this should include a policy for any samples sent away for further analysis. It should also include documented procedures for the management and reporting of incidents.

9 The laboratory must participate in an accredited External Quality Assessment Scheme (EQAS), appropriate for the DNA referrals for sickle cell and thalassaemia screening e.g. UK NEQAS, and must be able to demonstrate satisfactory performance as defined by the criteria specified by the EQA scheme organisers. It is expected that laboratories will participate specifically in UK NEQAS and will agree to share performance information with the Programme Centre.

10 Laboratories must have fail-safe arrangements in place. This includes checking all samples have been received and all results are received and acted upon (positive and negative) in a timely manner.

11 The laboratory must participate in audit at local, regional and national level, to assess the effectiveness of the national screening programme. This includes feedback of results to maternity and paediatric services to assist with local audits and to inform the national audit of prenatal diagnosis and review of antenatal screening.

12 The laboratory must be willing to release information on laboratory performance to any appropriate monitoring group of the National Screening Committee and the NHS Sickle Cell and Thalassaemia screening Programme Centre, and be open to peer review visits and inspection by the commissioners or their representatives at any reasonable time, by mutual agreement.
Family Origin Questionnaire

What are your family origins?

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

A. AFRICAN OR AFRICAN-CARIBBEAN (BLACK)
   - Woman
   - Baby’s father
   - Caribbean Islands
   - Africa (excluding North Africa)
   - Any other African or African-Caribbean family origins (please write in…)

B. SOUTH ASIAN (ASIAN)
   - Woman
   - Baby’s father
   - India or African-Indian
   - Pakistan
   - Bangladesh

C. SOUTH EAST ASIAN (ASIAN)
   - Woman
   - Baby’s father
   - China including Hong Kong, Taiwan, Singapore
   - Thailand, Indonesia, Burma
   - Malaysia, Vietnam, Philippines, Cambodia, Laos
   - Any other Asian family origins (please write in…)(e.g. Caribbean-Asian)

D. OTHER NON-EUROPEAN (OTHER)
   - Woman
   - Baby’s father
   - North Africa, South America etc
   - Middle East (Saudi Arabia, Iran etc)
   - Any other Non-European family origins (please write in…)

E. SOUTHERN & OTHER EUROPEAN (WHITE)
   - Woman
   - Baby’s father
   - Sardinia
   - Greece, Turkey, Cyprus
   - Italy, Portugal, Spain
   - Any other Mediterranean country
   - Albania, Czech Republic, Poland, Romania, Russia etc

F. UNITED KINGDOM (WHITE) refer to chart at the back
   - Woman
   - Baby’s father
   - England, Scotland, N Ireland, Wales

G. NORTHERN EUROPEAN (WHITE) refer to chart at the back
   - Woman
   - Baby’s father
   - Austria, Belgium, Ireland, France, Germany, Netherlands
   - Scandinavia, Switzerland etc
   - Any other European family origins, refer to chart (please write in)(e.g. Australia, N America, S Africa)

* Hb Variant Screening Requested by (F) and/or (G)
  # Higher risk for alpha zero thalassaemia

H. DON’T KNOW (incl. pregnancies with donor egg/sperm)
   - Woman
   - Baby’s father

I. DECLINED TO ANSWER

J. ESTIMATED DELIVERY DATE
   (please write in if not above)

K. GESTATION AT TIME OF TEST

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

Screening test declined
Do you want to give a reason why declined?
Yes

No

REPORT DESTINATION (e.g. Community Midwife, GP, Antenatal Clinic, Obstetrician)

All women need to be informed that routine analysis of blood may identify them as a thalassaemia carrier. In low prevalence areas OFFER haemoglobin variant screening to all women if they or the baby’s father have answers in any yellow box. In high prevalence areas OFFER haemoglobin variant screening to all women irrespective of answers, ie. if they or the baby’s father have answers in white and yellow boxes A - I.

Signed ___________________________ Print Name ___________________________ Job Title ___________________________ Date ___________________________

(by Health Care Professional Completing the Form)
Guidance for Health Care Professionals

Screening and Diagnostic Uses of the Family Origin Questionnaire

In low prevalence areas the Family Origin Questionnaire (FOQ) is principally used as a tool to identify women who are at highest risk of being a carrier or having a baby with a haemoglobin variant or disorder.

In high and low prevalence areas the FOQ is used as a tool by laboratory staff to help with the interpretation of results, particularly in the interpretation of results indicating possible alpha or beta thalassaemia. The family origin is also relevant in the interpretation of red blood cell indices and essential for accurate prenatal diagnosis. More information about its use can be found in the laboratory handbook: http://sct.screening.nhs.uk/publications

Therefore you need to ask for the family origins of both the woman AND the baby’s father going back at least 2 generations (or more if possible).

Women with Sickle Cell Disease

Screening will also identify women with sickle cell disease, who should be considered “high risk” requiring specialist care during pregnancy from an Obstetrician and Haematologist, and who should be booked for a hospital delivery.

“Low risk” Family Origins

People with family origins from the countries listed below are considered at low risk for haemoglobin variants.

United Kingdom (White)

England, Scotland, Northern Ireland, Wales.

Northern European (White)

Austria, Belgium, Denmark, Greenland, Iceland, Ireland (Eire), Finland, France, Germany, Luxembourg, Netherlands, Norway, Sweden, Switzerland.

Some populations of the following countries have Northern European origin (countries listed above) and are also at low risk for haemoglobin variants:

Northern European Origin (White)

Australia, North America (USA, Canada), South Africa, New Zealand.

Obtaining a supply of FOQ forms

You can obtain a supply of Family Origin Questionnaire forms from Prolog. Telephone 0300 123 1002

Code ANSPFQ 07/07
## Appendix 2 - Newborn screening status codes version 2

<table>
<thead>
<tr>
<th>Screening Status Code</th>
<th>Suggested term displayed in child health system</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Specimen received in laboratory</td>
<td>Same value applies to all screening tests (ie relates to the bloodspot card)</td>
</tr>
</tbody>
</table>
|                       | Additional data items to be provided with this status code and entered into Child Health systems. | Electronically or by manual means:  
  • Date sample taken  
  • Date sample received in laboratory  
  • Laboratory identifier |
| 02                    | (Condition Screened for) Declined             | Applies to each screening test individually |
| 03                    | (Condition Screened for) Repeat/Further sample required | Applies to each screening test individually. It is important for users of the child health system to understand that this status code indicates that there is not a screening outcome associated with the condition screened for on this sample.  
Two additional data items:  
“Reason for repeat test” and “Repeat test comment” are required where status code 03 applies.  
“Reason for repeat test” will include the following pick list for all conditions screened for:  
  • Too young for reliable screening  
  • Too soon after transfusion (<72 hours)  
  • Unsuitable sample  
  • Insufficient sample  
  • Unsatisfactory Analysis  
In addition “Reason for repeat test” will also include the following additional options for:  
Sickle:  
  • Transfusion  
  • Too premature for testing  
CHT  
  • Pre-term  
  • Borderline result  
CF  
  • Inconclusive  
PKU  
  • Borderline result  
  • Raised tyrosine  
“Repeat test comment”  
Free text field |
<table>
<thead>
<tr>
<th>Screening Status Code</th>
<th>Suggested term displayed in child health system</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>04</td>
<td>(Condition screened for) Not suspected</td>
<td>Applies to each screening test individually</td>
</tr>
<tr>
<td>05</td>
<td>(Condition screened for) Carrier</td>
<td>Applies to Sickle cell and Cystic Fibrosis screening tests. A free text comments field is required in association with this status code: “(Condition Screened for) Carrier comment”. This text field is required to enable additional information to be provided to Child Health if necessary.</td>
</tr>
<tr>
<td>06</td>
<td>Carrier of Other Haemoglobin</td>
<td>Applies to sickle cell disease screening test only</td>
</tr>
<tr>
<td>07</td>
<td>(Condition screened for) Not suspected Other Disorders Follow Up</td>
<td>Applies to each screening test individually</td>
</tr>
<tr>
<td>08</td>
<td>(Condition screened for) Suspected</td>
<td>Applies to each screening test individually</td>
</tr>
<tr>
<td>09</td>
<td>(Condition screened for) Not screened/ Screening incomplete*</td>
<td>Applies to each screening test individually. Additional data item required: “Reason for no result” will include the following pick list for all conditions screened for: Died, Unreliable result, Moved out of area. Additional value for CF only: Too old &gt;8 weeks**. **If no specimen is collected in this situation and it is a request for a second specimen (previous raised IRT) it is critical that a process is in place to ensure the baby is recalled for follow up.</td>
</tr>
<tr>
<td>10</td>
<td>HbS not suspected (by DNA) No other Hb/thal excluded</td>
<td></td>
</tr>
</tbody>
</table>

*this code applies to specimens collected and not analysed/result unreliable and in situations where a specimen is not collected
## Appendix 3 - Antenatal screening status codes

These status codes are not meant to replace the full laboratory report and are not designed to be used in detailed counselling of women and the fathers. They are to be used in conjunction with those reports to maternity departments to check that all their booking patients have an appropriate result; that all requests for repeats have been acted on and all baby's fathers invited for testing, as well as to facilitate linkage with the newborn screening results.

The examples of Hb phenotypes are just that: examples. It is not possible to give a definitive list when so many Hb variants exist.

<table>
<thead>
<tr>
<th>Code</th>
<th>Suggested term</th>
<th>Expanded comment</th>
<th>Examples of Hb phenotypes</th>
<th>Partner testing</th>
<th>Report format</th>
<th>Comments/Guidance for use</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not indicated</td>
<td>Testing not indicated by indices</td>
<td></td>
<td>No</td>
<td>0</td>
<td>This code is used by low prevalence labs only. It is for or FOQ women with MCH 27pg and above who are, and whose partners are in low risk categories on FOQ</td>
</tr>
<tr>
<td>1</td>
<td>No abnormality detected</td>
<td>Nothing abnormal detected on screening</td>
<td>AA</td>
<td>No</td>
<td>1</td>
<td>For women who have had laboratory testing for abnormal haemoglobins, and have had their Hb A₂ screening measured, and in whom nothing abnormal is detected</td>
</tr>
<tr>
<td>2</td>
<td>Non-significant carrier</td>
<td>Detail of Hb type</td>
<td>AGPhiladelphia, AJMellut</td>
<td>No</td>
<td>3</td>
<td>The majority of haemoglobins other than those listed under Code 5 will fall into this category, but you may need to seek advice if unsure</td>
</tr>
<tr>
<td>3</td>
<td>Iron Deficiency OR possible alpha thalassaemia (low risk)</td>
<td>alpha thalassaemia in low risk groups may be masked by iron deficiency (or with MCH&gt;25 pg)</td>
<td>α thalassaemia/iron deficiency from low risk group, or from a high risk group if the MCH is &gt;25 pg (probable α⁺)</td>
<td>No</td>
<td>7(a) or(b)</td>
<td>The code is about the general category of result, and the recommended action. Choose report format 7a or 7b as appropriate</td>
</tr>
<tr>
<td>4</td>
<td>Homozygote or compound heterozygote: neither of genetic significance</td>
<td>Detail of Hb type</td>
<td>D[::-]/[;]</td>
<td>No</td>
<td>3 (modified)</td>
<td>You will need to modify the non-significant carrier report format to indicate the homozygosity or compound heterozygosity</td>
</tr>
<tr>
<td>Code</td>
<td>Suggested term</td>
<td>Expanded comment</td>
<td>Examples of Hb phenotypes</td>
<td>Partner testing</td>
<td>Report format</td>
<td>Comments/Guidance for use</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>------------------</td>
<td>---------------------------</td>
<td>----------------</td>
<td>--------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>5</td>
<td>Significant carrier</td>
<td>Detail of Hb type</td>
<td>AS, AC, AD&lt;sup&gt;Punjab&lt;/sup&gt;, AE, AO&lt;sup&gt;Ale&lt;/sup&gt;, A&lt;sup&gt;Le&lt;/sup&gt;pore, &lt;br&gt;β thalassaemia trait, &lt;br&gt;δβ thalassaemia trait &lt;br&gt;or α thalassaemia/ &lt;br&gt;iron deficiency where &lt;br&gt;&lt;strong&gt;both&lt;/strong&gt; parents are &lt;br&gt;from high risk group</td>
<td>Yes</td>
<td>2, 4, 5 &lt;br&gt;6</td>
<td>Bear in mind there may be more than one genetic risk &lt;br&gt;for example a sickle carrier with MCH&lt;25 pg from a &lt;br&gt;high risk for alpha zero group and in this case you may &lt;br&gt;need to modify the significant carrier report formats &lt;br&gt;to indicate the multiple risk</td>
</tr>
<tr>
<td>6</td>
<td>Homozygote or compound heterozygote: &lt;br&gt;either or both of genetic significance</td>
<td>Detail of Hb type</td>
<td>CC, Cβ &lt;br&gt;thalassaemia, &lt;br&gt;DD-Punjab, EE</td>
<td>Yes</td>
<td>2, 4, 5 &lt;br&gt;or 6 (modified)</td>
<td>Bear in mind there may be more than one genetic risk &lt;br&gt;for example a patient with Hb Cβ thalassaemia and in &lt;br&gt;this case you may need to modify the significant carrier &lt;br&gt;report formats to indicate the multiple risk</td>
</tr>
<tr>
<td>7</td>
<td>Significant disorder</td>
<td>Detail of Hb type</td>
<td>SS, SC, SD, SE, &lt;br&gt;SO&lt;sup&gt;Arab&lt;/sup&gt;, &lt;br&gt;Sβ thalassaemia, &lt;br&gt;β thalassaemia major/intermedia</td>
<td>Yes</td>
<td>8</td>
<td>In these circumstances there are two considerations, the &lt;br&gt;mother’s health and the genetic risk</td>
</tr>
<tr>
<td>8</td>
<td>Repeat required State timing/ urgency of repeat</td>
<td>Reason for repeat.</td>
<td>Any variant or condition not yet identified, or where &lt;br&gt;an unsuitable sample has been received</td>
<td>Yes</td>
<td>Customised</td>
<td>Due to time constraints a partner will usually be requested in &lt;br&gt;these circumstances. Consider telephoning for repeat &lt;br&gt;samples to minimize delays</td>
</tr>
<tr>
<td>9</td>
<td>Result pending</td>
<td></td>
<td>Any variant or condition not yet identified</td>
<td>Yes</td>
<td>Customised</td>
<td>Due to time constraints a partner will usually be requested in &lt;br&gt;these circumstances. Issue an interim report (this may be by &lt;br&gt;telephone in the first instance)</td>
</tr>
<tr>
<td>10</td>
<td>Screening declined</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 4 - Outcome codes

The outcome codes are a series of codes that reflect the stage of each woman's/couple's journey along the screening pathway. It is anticipated that this code will give a picture of the couple's outcome following screening and what the possibilities are for the baby's result.

The codes may change during the current pregnancy and could also be different in each pregnancy.

These codes are meant to be cumulative in order to reflect the stage of each woman's/couple's journey along the screening pathway.

For example a carrier mother would start with a code of 5b, which might change to 5bc if the partner test reveals no abnormality, or 5bd for an at risk couple

<table>
<thead>
<tr>
<th>Code</th>
<th>Suggested term</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Screening of baby's father NOT indicated/NOT required</td>
</tr>
<tr>
<td>b</td>
<td>Screening of baby's father required</td>
</tr>
<tr>
<td>c</td>
<td>Baby's father has been tested and no genetic risk identified</td>
</tr>
<tr>
<td>d</td>
<td>At risk couple identified</td>
</tr>
<tr>
<td>e</td>
<td>Baby's father declined screening/baby's father unavailable for screening</td>
</tr>
<tr>
<td>f</td>
<td>Woman declined screening (baby's father not offered screening)</td>
</tr>
</tbody>
</table>

Appendix 5 - Laboratory criteria for analysing liquid capillary blood samples on babies born to known high risk couples (1 in 4 risk of a significant haemoglobinopathy)

Best Practice Guidelines

The Sickle Cell and Thalassaemia Screening Programme has been asked to produce best practice guidelines for those maternity services where laboratories are requested to analyse newborn liquid capillary blood specimens from known high risk pregnancies. To be a known ‘1 in 4’ high risk pregnancy, the haemoglobin results must be known on both parents. This service should be seen as a special ‘on-demand’ service to alleviate parental anxiety and not as a fail-safe or substitute for the newborn bloodspot screening programme, nor to gain a more rapid entry into clinical care. If local arrangements can be implemented to obtain fast track results from the routine bloodspot screening programme, then this approach should be encouraged. Those laboratories that perform second-line confirmatory testing for the newborn bloodspot screening programme would already satisfy the majority of these criteria.

Laboratory tests may be requested on newborn babies for clinical reasons but these best practice guidelines have been developed specifically for those babies born to known high risk couples. Specimens taken for other clinical diagnostic purposes fall outside these guidelines, although the requester may wish to assure themselves that the laboratory has sufficient competence and expertise to analyse specimens from neonates.

It is recognised that there is no ideal external quality assessment (EQA) scheme that tests proficiency with liquid blood specimens from neonates. Work has been undertaken with UK NEQAS (H) to provide these specimens for laboratories that analyse liquid blood and they are now included in the scheme.

1 The analytical technique for initial testing of the capillary specimen and the second line confirmatory test must be appropriate for specimens from the newborn period. Some analytical protocols are designed for testing adult blood and are not optimal for neonates.

2 Ideally the laboratory should participate in an appropriate UK national EQA scheme that assesses the analysis and interpretation of results from neonatal specimens. It is recognised that the present UK NEQAS (H) newborn sickle cell screening scheme uses dried bloodspots and that results may not be as reliable as with liquid specimens but enrolment in this scheme demonstrates a commitment to providing a quality analytical service for neonates.

3 The laboratory should analyse at least 20 specimens a year from babies aged up to three months. This is the minimum number of specimens deemed necessary to
obtain sufficient expertise and competence in the interpretation of results in neonates and infants. These specimens could include those received for reasons other than the pregnancy being at high risk.

4 Samples must be transported to the analysing laboratory in a reliable and swift manner, using a courier service if the specimen has to be referred, rather than risking delays using the postal service.

5 Both parents’ results must be reviewed before reporting the result of the liquid capillary sample. Since this is a service for pregnancies known to be at a 1 in 4 risk of a significant haemoglobinopathy, the requester must record the results of both parents on the request form.

6 The laboratory analysing the liquid specimen has the responsibility to make the result of the liquid capillary blood sample available to the newborn screening laboratory. There must be documented local systems in place for this process. Any discrepancies between the two results need to be investigated rapidly at local level by both laboratories and reported to the SCT Programme and regional team within one week of the discrepancy being detected.

7 Laboratories offering this service must collate data for audit purposes on the number of specimens tested, turnaround times and consistency with newborn screening results.

8 If the results from the liquid capillary specimen and the bloodspot screening programme are in accord with each other then a further confirmatory specimen is not needed.

9 The laboratory must have Standard Operating Procedures (SOPs) for the analytical methods and the rapid local reporting processes to the relevant healthcare professionals for the capillary specimen result. The SOP must include senior level checking of analytical results before reporting and follow up of the subsequent bloodspot screening result.

10 A mother and baby who are ready to be sent home should not be kept in hospital awaiting the results of these tests.

11 The laboratory must be CPA accredited.

Appendix 6 - Guidance concerning non-paternity

All genetic tests, including screening, undertaken during pregnancy and the newborn period will always encompass the possible issue of non-paternity. This needs to be considered when offering screening tests, and when inviting the woman’s partner or baby’s father for testing.

The role of health professionals is not to judge the situation but to provide clarity and discretion around genetic screening test results – particularly as there is no consensus on the rate of non-paternity in the population.

Obtaining this unsolicited information creates an ethical dilemma about whether to pass the information on, and to whom. Often the revelation of non-paternity may be detrimental to established relationships.

Antenatal screening

When a carrier woman is identified during antenatal screening, the issue of non-paternity should be considered when offering screening to the baby’s father. It should be highlighted to the mother that the correct person to be screened is the ‘baby’s father’ so that the pattern of genetic inheritance in the baby can be correctly assessed. When discussing results the need for discretion is essential.

Newborn screening for sickle cell diseases

Testing the newborn baby occasionally produces unexpected information (e.g. evidence of non-paternity), and health professionals must be alert to the need for discretion in pursuing family studies and in discussing results with the mother/parents.

Non-paternity may be suspected when

- a baby with a major haemoglobin disease has a carrier mother but the ‘father’ is not a carrier
- a baby identified as a carrier has neither their mother nor their father identified as a carrier.

However, even when screening results seem to suggest non-paternity, alternative
explanations must also be considered, for example:

- One parent carries a variant that cannot be detected by usual screening methods, e.g. an unstable variant or a silent form of β thalassaemia.
- There was an error
  a) with labelling a sample
  b) in the laboratory
  c) or in reporting the results.
- A parent’s identity may have been stolen by another person.
- The couple may have used assisted reproductive methods (artificial insemination by donor egg/sperm).
- The baby may have developed a de novo mutation, which although rare is possible.
- A very premature baby may not yet have developed any of their adult haemoglobin.

Guidance

Despite the above possibilities, the risk of non-paternity remains, and this needs to be handled carefully if relationships and family units are not to be disrupted. It is not in the interests of anyone (professional or parent) to cause a division in the relationship by revealing this information.

The professional needs to remain non-judgemental while considering the following actions:

1. Review the antenatal and newborn screening process to establish whether or not an error may have occurred at any stage of the pathway.
2. Explore the possibility of non-paternity with the mother, preferably on her own in private, without her partner present.
3. Agree with the baby’s mother how the situation will be dealt with if non-paternity is indeed a possibility.
4. Offer a re-test (in the first instance) to:
   a. mother
   b. baby.
5. If indicated, re-screen the father.
6. Carefully document results and communicate these only to those professionals who need the information to support the family.
Appendix 7 - Table of parental carrier state combinations that give rise to the risk of a fetus with significant sickle cell disease or β thalassaemia

(Table based on work of Prof. B. Modell)

### Mother

<table>
<thead>
<tr>
<th>Carrier of</th>
<th>Hb S</th>
<th>β thal</th>
<th>δβ thal</th>
<th>Hb Lepore</th>
<th>Hb E</th>
<th>Hb O^Arab</th>
<th>Hb C</th>
<th>Hb D^Punjab</th>
<th>HPFH</th>
<th>Not identified as a carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb S</td>
<td>Detroit</td>
<td>才会</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
</tr>
<tr>
<td>β thal</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
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</tr>
<tr>
<td>δβ thal</td>
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<td>热带</td>
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</tr>
<tr>
<td>Hb Lepore</td>
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<td>热带</td>
<td>热带</td>
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<td>热带</td>
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<td>Hb E</td>
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<td>热带</td>
<td>热带</td>
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<td>热带</td>
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</tr>
<tr>
<td>Hb O^Arab</td>
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<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
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<td>热带</td>
<td>热带</td>
<td>热带</td>
</tr>
<tr>
<td>Hb C</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
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<td>热带</td>
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</tr>
<tr>
<td>Hb D^Punjab</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
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<td>热带</td>
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<td>热带</td>
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</tr>
<tr>
<td>HPFH</td>
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<td>热带</td>
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<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
</tr>
<tr>
<td>Not identified as a carrier</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
<td>热带</td>
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</tr>
</tbody>
</table>

### Key:

- **Red**: Serious risk - refer couple for counselling - prenatal diagnosis to be offered
- **Light Red**: Less serious risk - refer couple for counselling - further investigation may be required
- **White**: Minimal risk
Appendix 8 - NHS Supply Chain National Framework Agreement for sickle cell and thalassaemia screening

The NHS Supply Chain framework agreement at present provides a list of suppliers of equipment and reagents for antenatal screening for sickle cell and thalassaemia and for newborn screening. The antenatal agreement also includes equipment and reagents for the measurement of Hb A1c used for monitoring diabetic patients. It is important to note that although the equipment and companies have satisfied the specifications stipulated in the tendering process, none of the equipment on the framework agreement has undergone a formal technical evaluation as part of this process. Separation and quantitation of haemoglobin variants and fractions varies between analysers. Laboratories must assure themselves that any analyser they choose is capable of providing accurate and reliable results for all of the screening specimens they receive. For antenatal screening the companies currently on the framework agreement are:- Bio-Rad Laboratories; Launch Diagnostics; Menarini Diagnostics; Sebia (UK); and Tosoh Bioscience. For newborn screening the companies are:- Bio-Rad Laboratories; Sebia (UK); Tosoh Bioscience and Trinity Biotech (UK Sales). This list may change as the contracts are retendered.

The companies have provided a comprehensive breakdown of the prices they will charge for different workload levels and different combination of tests. There are various options including leasing and capital purchase.

A laboratory is able to compare prices and equipment and make a decision about which supplier best suits their requirements. There is no desire to force laboratories in any particular direction. The advantages are that the prices are clear; the tendering process has been undertaken by the NHS Supply Chain and does not need to be gone through again. Contract performance will also be monitored. Laboratories are expected to use national framework agreements wherever possible.

Details should be available either directly from the local supplies department or from this contact at NHS Supply Chain:-

Katy Smith
Buyer – Pathology
NHS Supply Chain
Unit 5 Telford Court, Chester Gates
Dunkirk Trading Estate
Chester CH1 6LT
Tel: 01244 582823

www.supplychain.nhs.uk/product-areas/pathology/contract-categories/

The companies themselves will also be able to supply details of their prices for the agreement.
# Appendix 9 - Laboratories involved in newborn screening

<table>
<thead>
<tr>
<th><strong>Director / Contact</strong></th>
<th><strong>Phone, Fax &amp; Email</strong></th>
<th><strong>Alternative Laboratory Contact</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Jacqui Calvin&lt;br&gt;Neonatal Screening Service&lt;br&gt;Box 247&lt;br&gt;<strong>Addenbrooke's Hospital</strong>&lt;br&gt;Hills Road&lt;br&gt;Cambridge&lt;br&gt;CB2 2QQ</td>
<td>P: 01223 257 130&lt;br&gt;F: 01223 216 867&lt;br&gt;<a href="mailto:jacqui.calvin@addenbrookes.nhs.uk">jacqui.calvin@addenbrookes.nhs.uk</a></td>
<td>Sarah Hogg&lt;br&gt;P: 01223 596 172&lt;br&gt;<a href="mailto:sarah.hogg@addenbrookes.nhs.uk">sarah.hogg@addenbrookes.nhs.uk</a></td>
</tr>
<tr>
<td>Mr Paul Griffiths&lt;br&gt;Newborn Screening &amp;&lt;br&gt;Biochemical Genetics&lt;br&gt;<strong>Birmingham Children’s Hospital</strong>&lt;br&gt;Steelhouse Lane&lt;br&gt;Birmingham&lt;br&gt;B4 6NH</td>
<td>P: 0121 333 9923&lt;br&gt;F: 0121 333 9911&lt;br&gt;<a href="mailto:paul.griffiths@bch.nhs.uk">paul.griffiths@bch.nhs.uk</a></td>
<td>Ms Kate Hall&lt;br&gt;P: 0121 333 9903&lt;br&gt;F: 0121 333 9913&lt;br&gt;<a href="mailto:kate.hall@bch.nhs.uk">kate.hall@bch.nhs.uk</a></td>
</tr>
<tr>
<td>Mrs D Mantio / Ms L Fraser&lt;br&gt;Department of Haematology&lt;br&gt;<strong>Central Middlesex Hospital</strong>&lt;br&gt;Acton Lane, Park Royal&lt;br&gt;London&lt;br&gt;NW10 7NS</td>
<td>P: 020 8453 2671&lt;br&gt;F: 020 8453 2581&lt;br&gt;<a href="mailto:deborah.mantio@nhs.net">deborah.mantio@nhs.net</a>&lt;br&gt;<a href="mailto:laurafraser2@nhs.net">laurafraser2@nhs.net</a></td>
<td>P: 020 8453 2671&lt;br&gt;F: 020 8453 2581&lt;br&gt;<a href="mailto:nih-tr.cmhhbline@nhs.net">nih-tr.cmhhbline@nhs.net</a></td>
</tr>
<tr>
<td>Dr Niki Meston&lt;br&gt;Dept of Chemical Pathology&lt;br&gt;<strong>St Helier Hospital</strong>&lt;br&gt;Wrythe Lane, Carshalton&lt;br&gt;Surrey,&lt;br&gt;SM5 1AA</td>
<td>P: 020 8296 2660&lt;br&gt;F: 020 8296 3459&lt;br&gt;<a href="mailto:niki.meston@esth.nhs.uk">niki.meston@esth.nhs.uk</a>&lt;br&gt;Deputy Director: Dr Mary Clarke&lt;br&gt;P: 020 8296 2816&lt;br&gt;<a href="mailto:mary.clarke@esth.nhs.uk">mary.clarke@esth.nhs.uk</a></td>
<td>Mr Stephen Wilkins&lt;br&gt;P: 020 8296 2991&lt;br&gt;F: 020 8296 3459&lt;br&gt;<a href="mailto:stephen.wilkins@esth.nhs.uk">stephen.wilkins@esth.nhs.uk</a>&lt;br&gt;Ms Sarah Brown&lt;br&gt;<a href="mailto:sarah.brown@esth.nhs.uk">sarah.brown@esth.nhs.uk</a>&lt;br&gt;P: 020 8296 2812</td>
</tr>
<tr>
<td>Prof Simon Heales&lt;br&gt;Newborn Screening Laboratory&lt;br&gt;<strong>Great Ormond Street Hospital</strong>&lt;br&gt;5th Floor;&lt;br&gt;Camelia Botnar Laboratories&lt;br&gt;Great Ormond Street&lt;br&gt;London&lt;br&gt;W1N 3JH</td>
<td>P: 020 7829 8642&lt;br&gt;<a href="mailto:simon.heales@gosh.nhs.uk">simon.heales@gosh.nhs.uk</a></td>
<td>Dr Katie Harvey&lt;br&gt;P: 020 7405 7843&lt;br&gt;F: 020 7829 8624&lt;br&gt;<a href="mailto:katie.harvey@gosh.nhs.uk">katie.harvey@gosh.nhs.uk</a></td>
</tr>
<tr>
<td>Fiona Carragher&lt;br&gt;Biochemical Sciences&lt;br&gt;4th Floor North Wing&lt;br&gt;<strong>St Thomas’ Hospital</strong>&lt;br&gt;SE1 7EH</td>
<td>P: 020 7188 1283&lt;br&gt;F: 020 7188 1269&lt;br&gt;<a href="mailto:fiona.carragher@gsts.com">fiona.carragher@gsts.com</a></td>
<td>Wyn Griffiths&lt;br&gt;P: 020 7188 1267&lt;br&gt;<a href="mailto:wyn.griffiths@gsts.com">wyn.griffiths@gsts.com</a></td>
</tr>
<tr>
<td>Director / Contact</td>
<td>Phone, Fax &amp; Email</td>
<td>Alternative Laboratory Contact</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td><strong>Dr David Rees</strong></td>
<td>P: 020 3299 3242</td>
<td>Chris Lambert</td>
</tr>
<tr>
<td>The Red Cell Centre</td>
<td>F: 020 3299 1035</td>
<td>P: 020 3299 2455</td>
</tr>
<tr>
<td>King’s College Hospital</td>
<td><a href="mailto:david.rees2@nhs.net">david.rees2@nhs.net</a></td>
<td>F: 020 3299 1267</td>
</tr>
<tr>
<td>Denmark Hill</td>
<td></td>
<td><a href="mailto:chris.lambert@nhs.net">chris.lambert@nhs.net</a></td>
</tr>
<tr>
<td>London SE5 9RS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dr David Sinclair</strong></td>
<td>P: 02392 286 812</td>
<td>Vivienne Laakvand</td>
</tr>
<tr>
<td>Blood Sciences Laboratory</td>
<td>F: 02392 286 349</td>
<td>P: 02392 286 903</td>
</tr>
<tr>
<td>Queen Alexandra Hospital</td>
<td>david.sinclair@port Hosp.nhs.uk</td>
<td>vivienne.laakvand@port Hosp.nhs.uk</td>
</tr>
<tr>
<td>Cosham, Portsmouth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hants, PO6 3LY</td>
<td></td>
<td>Toby Greenfield</td>
</tr>
<tr>
<td><strong>Mr Paul Newland</strong></td>
<td>P: 0151 252 5486</td>
<td>P: 02392 286000 ext 1765</td>
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<tr>
<td>Clinical Biochemistry</td>
<td>F: 0151 282 4613</td>
<td>toby.greenfield@port Hosp.nhs.uk</td>
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<tr>
<td>Royal Liverpool Children’s Hospital</td>
<td><a href="mailto:paul.newland@alderhey.nhs.uk">paul.newland@alderhey.nhs.uk</a></td>
<td></td>
</tr>
<tr>
<td>Eaton Road</td>
<td></td>
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<tr>
<td>Liverpool, L12 2AP</td>
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<tr>
<td><strong>Mrs Lesley Tetlow</strong></td>
<td>P: 0161 701 1268</td>
<td>Deputy: Beverly Hird</td>
</tr>
<tr>
<td>Department of Biochemistry</td>
<td>P: 0161 701 5167</td>
<td>P: 0161 701 2265</td>
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<tr>
<td>Royal Manchester Children’s Hospital</td>
<td><a href="mailto:lesley.tetlow@cmft.nhs.uk">lesley.tetlow@cmft.nhs.uk</a></td>
<td><a href="mailto:beverly.hird@cmft.nhs.uk">beverly.hird@cmft.nhs.uk</a></td>
</tr>
<tr>
<td>Oxford Road</td>
<td></td>
<td></td>
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<tr>
<td>Manchester M13 9WL</td>
<td></td>
<td>Laura Hamilton</td>
</tr>
<tr>
<td><strong>Dr Kim Bartlett</strong></td>
<td>P: 0191 282 4566</td>
<td>Deputy: Catherine Collingwood</td>
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<tr>
<td>Biochemistry/</td>
<td><a href="mailto:kim.bartlett@nuth.nhs.uk">kim.bartlett@nuth.nhs.uk</a></td>
<td><a href="mailto:catherine.collingwood@alderhey.nhs.uk">catherine.collingwood@alderhey.nhs.uk</a></td>
</tr>
<tr>
<td>Haematology Departments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Floor, William Leech Building</td>
<td></td>
<td></td>
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<tr>
<td>Royal Victoria Infirmary</td>
<td></td>
<td></td>
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<tr>
<td>Queen Victoria Road</td>
<td></td>
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<tr>
<td>Newcastle-Upon-Tyne NE1 4LP</td>
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<tr>
<td><strong>Dr M J Henderson</strong></td>
<td>P: 0113 206 6861</td>
<td>Lisa Thomas</td>
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<tr>
<td>Biochemical Genetics</td>
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<td>P: 0191 282 4761</td>
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<td>Block 46</td>
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<td><a href="mailto:lisa.thomas@nuth.nhs.uk">lisa.thomas@nuth.nhs.uk</a></td>
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<td>St. James’s University Hospital</td>
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<tr>
<td>Leeds</td>
<td></td>
<td>Janice Dunn</td>
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<tr>
<td>Yorkshire LS9 7TF</td>
<td></td>
<td>T. 0191 282 0339</td>
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<tr>
<td><strong>Dr Leonie Shapiro</strong></td>
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<td>F: 0191 282 4217</td>
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<tr>
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<tr>
<td><strong>Mr Daniel Herrera</strong></td>
<td>P: 0113 206 6860</td>
<td>Daniel Herrera</td>
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<tr>
<td><strong>Mr Tim Williams</strong></td>
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<td>Daniel Herrera</td>
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<td>0113 206 4881</td>
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<td>Daniel Herrera</td>
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<td><strong>Mrs Lisa Farrar</strong></td>
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<td><a href="mailto:timothy.williams@leedst.nhs.uk">timothy.williams@leedst.nhs.uk</a></td>
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<tr>
<td><strong>Director / Contact</strong></td>
<td><strong>Phone, Fax &amp; Email</strong></td>
<td><strong>Alternative Laboratory Contact</strong></td>
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<tr>
<td><strong>Dr Jim Bonham</strong></td>
<td>P: 0114 271 7404</td>
<td>Ms Melanie Downing</td>
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<tr>
<td>Regional Newborn Screening Service</td>
<td><a href="mailto:jim.bonham@sch.nhs.uk">jim.bonham@sch.nhs.uk</a></td>
<td>P: 0114 271 7302</td>
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<td><a href="mailto:melanie.downing@sch.nhs.uk">melanie.downing@sch.nhs.uk</a></td>
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<td>Sheffield Children’s Hospital (NHS) FT</td>
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<td>Joyce Baston</td>
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<td>P: 0114 271 7500</td>
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<td></td>
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<td><a href="mailto:joyce.baston@sch.nhs.uk">joyce.baston@sch.nhs.uk</a></td>
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<td><strong>Dr Helena Kemp</strong></td>
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<td>Ann Brown</td>
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<td>Department Clinical Biochemistry</td>
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<td>The Pathology Sciences Laboratory</td>
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<td>Sharon Evans</td>
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<tr>
<td>Westbury-on-Trym</td>
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<td>P: 0117 323 8353</td>
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<tr>
<td>Bristol BS10 5NB</td>
<td><a href="mailto:helenakemp@nbt.nhs.uk">helenakemp@nbt.nhs.uk</a></td>
<td><a href="mailto:sharon.evans@nbt.nhs.uk">sharon.evans@nbt.nhs.uk</a></td>
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<tr>
<td><strong>Professor Jonathan Kay</strong></td>
<td>P: 01865 220470</td>
<td>Ian Smith</td>
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<tr>
<td>Oxford Screening Laboratory</td>
<td></td>
<td>P: 01865 220488</td>
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<tr>
<td>Clinical Biochemistry</td>
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<td>John Radcliffe Hospital</td>
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<td><a href="mailto:jonathan.kay@ouh.nhs.uk">jonathan.kay@ouh.nhs.uk</a></td>
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<td>OX3 9DU</td>
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<tr>
<td><strong>Dr Shirley Henderson</strong></td>
<td>P. 01865 572769</td>
<td>Alice Gallienne</td>
</tr>
<tr>
<td>National Haemoglobinopathy Reference Laboratory</td>
<td></td>
<td>P: 01865 572769</td>
</tr>
<tr>
<td>Haematology, Level 4</td>
<td>F. 01865 572775</td>
<td><a href="mailto:alice.gallienne@ouh.nhs.uk">alice.gallienne@ouh.nhs.uk</a></td>
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<tr>
<td>John Radcliffe Hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxford</td>
<td><a href="mailto:shirley.henderson@ouh.nhs.uk">shirley.henderson@ouh.nhs.uk</a></td>
<td></td>
</tr>
<tr>
<td>OX3 9DU</td>
<td></td>
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</tr>
</tbody>
</table>
Appendix 10 - Laboratories undertaking prenatal diagnosis (PND) and pre-implantation genetic diagnosis (PGD)

John Radcliffe Hospital, Oxford
Provides DNA Testing only
Address National Haemoglobinopathy Reference Laboratory
Molecular Haematology
Level 4
John Radcliffe Hospital
Oxford
OX3 9DU
Contact Dr Shirley Henderson, Principal
Clinical Scientist/Deputy Director
Tel 01865 572769
Fax 01865 572775
Email hbopathy.screening@nhs.net;
molhaem@ouh.nhs.uk
Website www.oxfordradcliffe.nhs.uk

King's College Hospital, London
Provides DNA Testing and counselling
Address: Red Cell Centre
1st Floor Bessemer Wing
King’s College Hospital
Denmark Hill
London SE5 9RS
Contact Dr Barnaby Clark,
Principal Clinical Scientist
Tel 020 3299 4337
Tel lab 020 3299 2265
Fax 020 3299 1035
Email barnaby.clark@nhs.net
Website kingspath.co.uk/

University College Hospital, London
Provides fetal sampling, DNA testing, counselling and pre-implantation genetic diagnosis (PGD)
Address Haemoglobinopathy Genetics Centre
86-96 Chenies Mews
London WC1E 6HX
Contact Dr Mary Petrou, Director
Email mary.petrou@uclh.nhs.uk;
haem.gen@uclh.nhs.uk
Tel 020 3447 9458
Fax 020 3447 9864

Guy’s Hospital, London
Provides pre-implantation genetic diagnosis (PGD) for haemoglobinopathy
Address Guy’s & St Thomas’ Centre for Pre-implantation Genetic Diagnosis Assisted Conception Unit,
4th Floor Thomas Guy House
Guy’s Hospital, St Thomas Street,
London SE1 9RT
Contact
Tel 020 7811 0504,
Fax 020 7188 0490
Email pgd@kcl.ac.uk
### Appendix 11 - Proposed networks of clinical care

<table>
<thead>
<tr>
<th>Region</th>
<th>Newborn Screening Lab</th>
<th>SHT Network Centres with associated local hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>East of England</td>
<td>Cambridge, Great Ormond Street, Central Middlesex</td>
<td>East of England Trusts now linked into North Middlesex London network E&amp;N Herts (Lister &amp; QEII); West Essex (PAH, Harlow) (choice given); Norfolk, Suffolk, Peterborough &amp; Cambridge units (satellite clinic held at Addenbrooke’s and discussions to set one up in Norwich)</td>
</tr>
<tr>
<td>East Midlands</td>
<td>Sheffield</td>
<td>Nottingham, Leicester Associated local hospitals: Derby, Kettering and Northampton (adults)</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East London &amp; Essex</td>
<td>Great Ormond Street, Central Middlesex</td>
<td>The Royal London Associated local hospitals: Barking Havering &amp; Redbridge, Whipp Cross Hospital, Newham, Homerton, Basildon, SE Essex (Southend); NE Essex (Colchester); Mid-Essex (Chelmsford); West Essex (choice given)</td>
</tr>
<tr>
<td>North East London</td>
<td>Great Ormond Street, Central Middlesex</td>
<td>North Middlesex Hospital/Great Ormond Street Associated local hospitals: Barnet, Chase Farm at Enfield, Princess Alexandra at Harlow, Addenbrooke’s at Cambridge, King’s Lynn, Norfolk and Norwich, Ipswich</td>
</tr>
<tr>
<td>North Central London</td>
<td>Great Ormond Street, Central Middlesex</td>
<td>University College Hospital/Whittington Hospital Links to many trusts particularly for tertiary thalassaemia review</td>
</tr>
<tr>
<td>North West London</td>
<td>Great Ormond Street, Central Middlesex</td>
<td>Central Middlesex, Imperial (St Mary's Hospital &amp; Hammersmith) Associated local hospitals: Ealing Hospital, West Middlesex at Isleworth, Hillingdon Hospital, Northwick Park, Chelsea and Westminster, plus outside the Brent area Luton, Milton Keynes, Watford and Bedford, Wycombe</td>
</tr>
<tr>
<td>South London</td>
<td>King's/GSTT</td>
<td>King’s College Hospital, London Guy’s and St Thomas’s Hospital (GSTT) Associated local hospitals: Croydon (paeds), South London Hospitals, Lewisham, St Thomas, Brighton and other SE Coast hospitals including Medway, Darent Valley, Some patients from: SW Essex and SE Essex</td>
</tr>
<tr>
<td>South West London</td>
<td>St Helier</td>
<td>St George’s Hospital Associated local hospitals: St Helier, Croydon (mainly adults), Kingston, Royal Surrey at Guilford, East Surrey at Redhill, St Peter’s Hospital at Chertsey</td>
</tr>
<tr>
<td>Northeast and Yorkshire &amp; Humberside</td>
<td>Newcastle, Leeds, Sheffield</td>
<td>St. James’ Hospital, Leeds Sheffield Associated local hospitals: Bradford, South Tees (James Cook Hospital) plus Newcastle &amp; NE</td>
</tr>
<tr>
<td>North West</td>
<td>Manchester, Liverpool</td>
<td>Manchester Children’s Hospital Alder Hey Children’s Hospital Associated local hospitals: Blackburn (Queen’s Park), Lancaster, Tameside</td>
</tr>
<tr>
<td>South Central</td>
<td>Oxford, Portsmouth</td>
<td>Configuration yet to be confirmed Hospitals in Region: - Royal Berkshire Hospital at Reading, The John Radcliffe Hospital at Oxford, Southampton (including Basingstoke and Portsmouth)</td>
</tr>
<tr>
<td>South East Coast</td>
<td>Various</td>
<td>Some trusts linked to South London via King’s or GSTT, other linked to South west London via St George’s</td>
</tr>
<tr>
<td>South West</td>
<td>Bristol</td>
<td>Bristol Royal Infirmary Local: Derriford Hospital, Plymouth (low prevalence area)</td>
</tr>
<tr>
<td>West Midlands</td>
<td>Birmingham</td>
<td>Birmingham Children’s Associated local hospitals: Sandwell, Wolverhampton, Coventry, University Hospital of N. Staffs at Stoke, (Northampton and Kettering paeds)</td>
</tr>
</tbody>
</table>
Appendix 12 - Useful organisations and websites

UK National Screening Committee
Imperial College Healthcare NHS Trust
Mint Wing, Centre Block G
South Wharf Road, London W2 1NY
Phone: 020 33126927
Website: www.screening.nhs.uk

NHS Sickle Cell and Thalassaemia Screening Programme
Division of Health and Social Care Research
King’s College School of Medicine
7th Floor, Capital House
42 Weston Street
London SE1 3QD
Tel 020 7848 6634
Fax 020 7848 6620
Email haemscreening@kcl.ac.uk
Website sct.screening.nhs.uk

Newborn Bloodspot Programmes
Great Ormond Street Hospital for children NHS Trust
Great Ormond Street
London, WC1N 3JH
Tel 020 7829 7884
Fax 020 78297881
Website newbornbloodspot.screening.nhs.uk

Sickle Cell Society
54 Station Road, Harlesden
London NW10 4UA
Phone 020 8961 7795
Fax 020 8961 8346
Email info@sicklecellsociety.org
Website www.sicklecellsociety.org

Sickle and Thalassaemia Association of Counsellors (STAC)
South West London Sickle Cell and Thalassaemia Centre,
Balham Health Centre, 120 Bedford Hill
Balham, London, SW12 9HP
Tel 020 8700 0615
Fax 020 8700 0634
Email info@stac.org
Website www.stacuk.org

UK Thalassaemia Society
19 The Broadway, Southgate
London N14 6PH
Phone 020 8882 0011
Fax 020 8882 8618
Email office@ukts.org
Website www.ukts.org

The North of England Bone Marrow and Thalassaemia Association
352 Oxford Road
Junction off Denmark Rd
Manchester M13 9NL
Phone 0161 2737 200
Fax 0161 2737 200

Sickle Cell and Thalassaemia Screening – Laboratory Support Service
Oxford University Hospitals
Designated Telephone line: 01865 572 767
Secure email lab.support@nhs.net
Secure fax 01865 572 775

Useful Websites

APOGI (Accessible publishing of genetic information)
www.chime.ucl.ac.uk/APoGI

British Committee for Standards in Haematology
www.bcshguidelines.com

Department of Health
www.dh.gov.uk

Expert Patients Programme
www.expertpatients.co.uk

NHS: about patient choice.
www.nhs.uk/choices

PEGASUS training materials
www.pegasus.nhs.uk

Royal College of Paediatrics and Child Health
www.rcpch.ac.uk

Brent Sickle Cell & Thalassaemia Centre
www.sickle-thal.nw lh.nhs.uk

Unit for the Social Study of Thalassaemia and sickle cell (TASC)
www.tascunit.com
Contact us

The NHS Sickle Cell and Thalassaemia Screening Programme

Tel: 020 7848 6634
Email: haemscreening@kcl.ac.uk
Web: sct.screening.nhs.uk