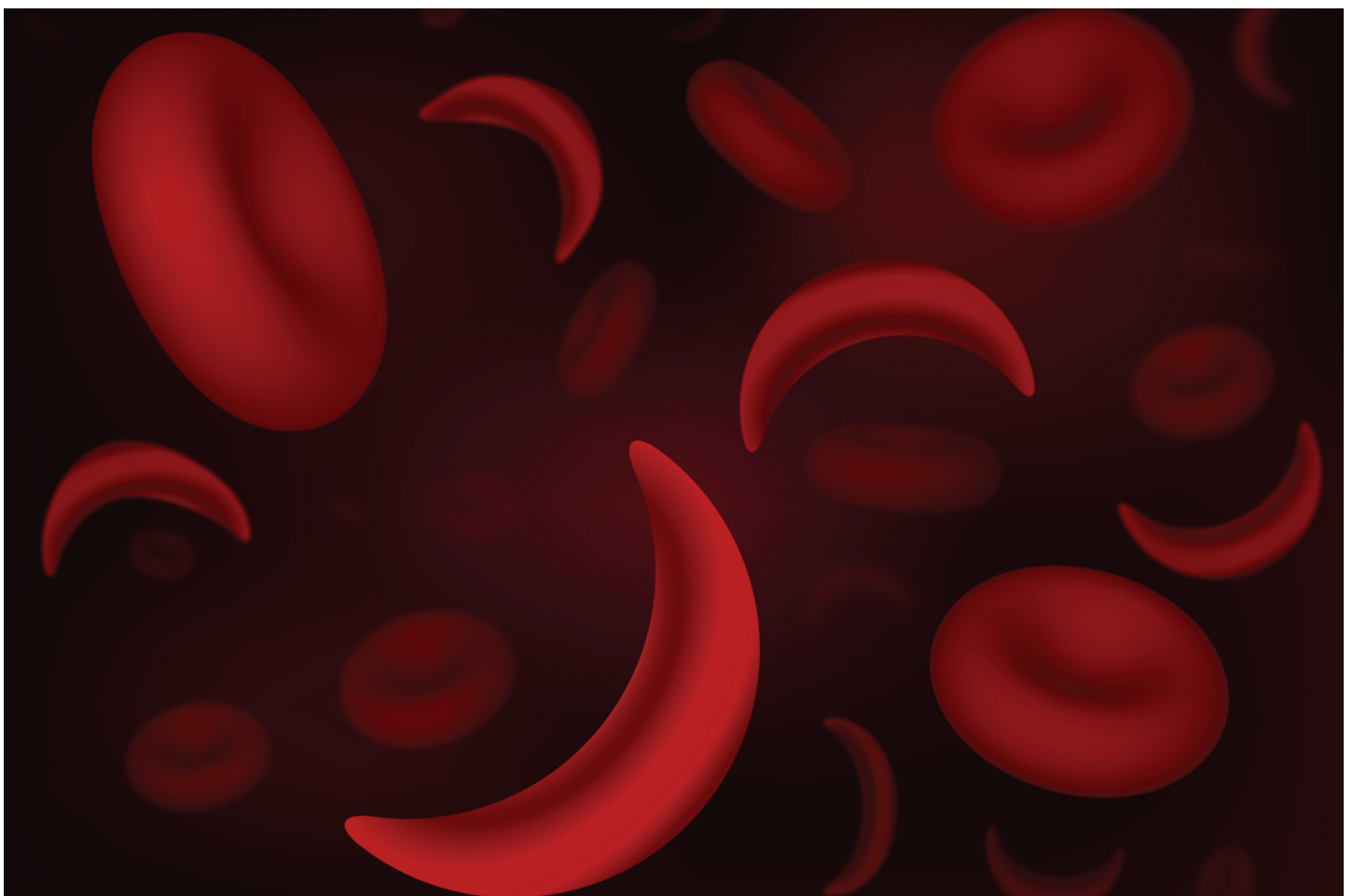




Public Health
England

NHS Sickle Cell and Thalassaemia Screening Programme

Handbook for antenatal laboratories



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Public Health England leads the NHS Screening Programmes

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Introduction

This is the fourth edition of the laboratory handbook, updating the edition published in October 2012. This handbook is for staff working in screening laboratories. It describes the policies and guidance that set out the requirements for laboratories involved in antenatal screening and DNA referral. Information for laboratories is regularly updated on GOV.UK^[1] and laboratories must check this section frequently. The policies described relate to a screening programme, not a diagnostic service.

Providers must comply with the national guidance for the management of safety concerns and incidents in screening programmes and NHS England guidance for the management of serious incidents^[2].

The British Committee for Standards in Haematology (BCSH) has published guidance about screening^[3].

We would like to thank all those who have contributed to the handbook, which has been produced in accordance with the PHE style guide.

If you wish to reference or acknowledge this document, please use the following format: NHS Sickle Cell and Thalassaemia Screening Programme, Handbook for Laboratories, 4th edition, September 2017.

At the time of going to print, all information and contact details were correct. Please note this handbook is available on GOV.UK^[1].

Haemoglobinopathies

The haemoglobinopathies are a heterogeneous group of more than 1,000 mutations, which are categorised into 2 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. The thalassaemias arise from inadequate production of structurally normal globin. There are also thalassaemic haemoglobinopathies that are produced when a structurally abnormal haemoglobin is synthesised at a reduced rate, for example HbE. Simple biochemical procedures can easily detect some of the abnormalities, but 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; so all individuals are theoretically at risk. It is not unusual for people to inherit more than one haemoglobin abnormality and many populations are at risk of a range of affected genes. Many haemoglobin mutations have no associated clinical significance. Others are associated with severe morbidity and mortality, most notably sickle cell disease and beta (β) thalassaemia major. Carriers are usually asymptomatic.

Sickle cell disease

Sickle haemoglobin (HbS) is a haemoglobin variant in which valine replaces glutamic acid, which is the sixth amino acid in the β globin chain. Other much rarer haemoglobins have been reported that have this same glutamic acid to valine substitution, but also an additional substitution in cis. All these variants will cause sickle cell disease in the situations described below for HbS.

Sickle cell disease results from the inheritance of certain genotypes, including:

- homozygosity for HbS (sickle cell anaemia)
- compound heterozygosity for HbS and an interacting mutation, such as HbC (Hb SC disease)
- β thalassaemia (Hb S/ β thalassaemia)

The sickling disorders are associated with severe life-threatening vaso-occlusive crises, overwhelming sepsis, splenic sequestration, aplastic crises, stroke, priapism, pulmonary hypertension, proliferative retinopathy, chronic organ damage and avascular necrosis of the hips and shoulders.

Beta thalassaemia major

Beta thalassaemias are a group of hereditary disorders that result in reduced (β^+) or absent (β^0) synthesis of β globin chains. These are an essential component of the main adult haemoglobin, HbA. Beta thalassaemias are usually caused by point mutations, small insertions or deletions and less frequently large deletions of the β globin gene located on chromosome 11. Inheritance is predominantly autosomal recessive although some dominant mutations have been reported. Mutation type and inheritance patterns result in variable phenotypes, which range from severe anaemia to clinically asymptomatic carriers. Thalassaemia major patients require lifelong transfusion therapy. This therapy has associated complications of iron overload, including endocrine dysfunction, cardiomyopathy, liver fibrosis and cirrhosis. A demanding daily regime of therapy with iron chelators that enable iron excretion from the body is required to treat these complications. Insufficient red cell replacement results in symptoms of anaemia, jaundice, poor growth and muscular development and skeletal changes from expansion of the bone marrow due to extramedullary haemopoiesis. Thalassaemia intermedia is a highly variable condition. Although some cases are transfusion-dependent it is usually a less severe disease than β thalassaemia major.

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 provides a support service for screening laboratories via designated telephone helplines and secure email. PHE funds the service and supports both antenatal and newborn screening enquiries.

Telephone: 01865 572 767

Email: lab.support@nhs.net

FAX: 01865 572 775

Antenatal screening

The aim of the antenatal screening programme is to offer timely antenatal sickle cell and thalassaemia screening to all women (and couples), to enable informed decision-making. We aim to:

- screen women by 10 weeks + 0 days gestation
- offer prenatal diagnosis (PND) to at risk women and couples by 12 weeks + 0 days gestation
- perform PND before 12 weeks + 6 days

Testing in subsequent pregnancies

If a woman is booked for antenatal care for a subsequent pregnancy, the healthcare professional must complete the [Family Origin Questionnaire \(FOQ\)](#), irrespective of previous screening. If the woman consents to screening, the healthcare professional must take the blood tests. They must ensure the sample is clearly labelled and sent to the laboratory accompanied by the completed [FOQ](#). This blood sample must be tested according to the algorithm, however, if on repeat testing a variant with identical characteristics to one previously identified and confirmed is detected the result from the first line screen is sufficient.

If a carrier or affected woman is identified, the baby's biological father must be offered a screening test, irrespective of previous screening history (Box 1).

If it is not possible to test the baby's biological father in every pregnancy and a previous result is being used then this fact must be recorded in the woman's notes for the current pregnancy.

The previous result must be from a laboratory accredited by the UK Accreditation Service (UKAS), and be consistent, unequivocal, well documented and interpreted and reported with the algorithms in this handbook.

Due to the complexities of the testing algorithm, screening for sickle cell and thalassaemia must be repeated in every pregnancy. Laboratories must be aware that serious incidents have resulted from failure to follow this policy.

Provision of screening

There are 2 approaches to the provision of the screening programme. The approach depends on whether a trust is defined as high prevalence or low prevalence. Each laboratory must use only one screening algorithm. Where high and low prevalence

trusts merge the high prevalence algorithm should be used. In all cases a failsafe system must be in place to make sure all eligible women have been offered screening, and those that accept are tested.

High prevalence

Trusts are considered high prevalence if 2% or more of the booking bloods are screen positive. The high prevalence algorithm is viewed as the gold standard. In this case, laboratory testing for haemoglobin variants and thalassaemia must be carried out on all women who have accepted screening (see pages [19](#) and [20](#)).

Low prevalence

Trusts are considered low prevalence where less than 1% of the booking bloods received by the laboratory are screen positive. Areas defined as low prevalence must perform screening for thalassaemia on all women who have accepted screening using the routine Hb and red cell indices. Further laboratory testing must be carried out on all women with defined abnormalities of the full blood count; those with high-risk family origins in either biological parent as determined by the [FOQ](#) and those women who request testing (see pages [21](#) and [22](#)).

Intermediate prevalence

We advise trusts that have a prevalence between these 2 values to continue to use their current algorithm.

Organisation of screening services

Providers must have a risk assessment of the entire pathway and protocols defining where responsibility falls for all aspects of the process. Laboratories must have protocols detailing receipt of samples, analytical procedures and reporting of results in a timely manner. If some or all samples are referred to or received from other laboratories, there must be service level agreements covering all of these aspects. Laboratory staff must follow the analytical guidelines, algorithms and service specifications produced by the NHS Sickle Cell and Thalassaemia Screening Programme. Additionally, there must be an agreement outlining who is responsible for the provision of key performance indicators (KPI), annual return data and data returns to National Congenital and Rare Disorders Registration Service.

Where opinions on results obtained are sought from third parties, laboratories must ensure they meet and comply with screening programme requirements. This is particularly important when using any service provided by a commercial organisation. When reports received from third parties are transcribed into internal

laboratory information systems, a full and exact copy of the report must be made. The transcribed report must be checked by an appropriate second person to ensure accuracy. Laboratories should scan these reports if possible.

Family origin questionnaire

The **FOQ** is used in both low and high prevalence areas. In low prevalence areas it is the basis for determining which women must be tested for haemoglobin variants. The woman's sample must be tested if she or the baby's biological father is in a high risk group. In high prevalence areas all consenting women must be tested. If a woman has declined screening then testing should not be carried out and this must be documented.

All women must be screened for thalassaemia using the mean cell haemoglobin (MCH) measurement, which has been shown to be stable for up to 4 to 5 days at room temperature^[4,5]. The family origin also assists laboratories with the interpretation of laboratory screening results, particularly the interpretation of results indicating possible alpha (α) thalassaemia. People originating from certain areas of the Far East and Eastern Mediterranean are at higher risk of α^0 thalassaemia, which in the homozygous state results in hydrops fetalis. Local policy must be in place for situations where an **FOQ** has not been received or is incomplete to ensure test reporting is not delayed. The **FOQ** must not be rejected if the only missing information is the gestational age.

The **FOQ** was developed from the initial literature review and research, funded by the programme centre. It was 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 and are different from the census based self-assigned ethnic group questions. 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 **FOQ** data fields must be consistent with the current version.

Situations requiring particular care

Fertility treatment – donor gametes

If the pregnancy has been achieved using a donor egg then the screening results on the woman will not be informative. The baby's biological father must be tested and if screen positive the report must recommend that the fertility clinic is contacted to obtain the biological mother's haemoglobinopathy results.

If donor sperm has been used and the woman has a positive screening result then the report must recommend that the fertility clinic is contacted to obtain the biological father's haemoglobinopathy results.

In the case of surrogacy the report must recommend that the fertility clinic is contacted to obtain haemoglobinopathy results of both biological parents.

It is best practice to test the pregnant woman in all circumstances to ensure optimal maternal care. The report must provide the appropriate advice for the circumstances of the pregnancy.

Adoption

If either biological parent has been adopted, the [FOQ](#) information may not accurately reflect the true family origins. Such cases must be treated as high risk and have full laboratory screening.

Bone marrow transplants

Where either biological parent has had a bone marrow transplant (BMT) it is likely the results obtained will reflect the BMT donor and will not accurately represent the genetic status of the fetus. If the biological mother has had a BMT, the baby's biological father must be tested to ensure this is not a high risk pregnancy. If DNA confirmation of the biological mother's status is required or if the baby's biological father is post BMT and requires testing, then pre-transplant DNA or DNA obtained from hair follicles should be used.

Box 1: Maternal conditions requiring testing of the baby's biological father.

Significant maternal haemoglobinopathies

The following maternal haemoglobinopathies should be detected by antenatal screening and are important for maternal care:

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

Carrier states in biological mother:

- HbS
- HbC
- HbD^{Punjab}
- HbE
- HbO^{Arab}
- Hb Lepore
- β thalassaemia
- $\delta\beta$ thalassaemia
- α^0 thalassaemia ($--/\alpha\alpha$)
- Hereditary Persistence of Fetal Haemoglobin (HPFH)

Any compound heterozygote state including one or more of the above conditions.
Any homozygous state of the above conditions.

Laboratory screening techniques

In low prevalence areas the first-line screen for thalassaemia is the full blood count.

The following techniques are suitable for use in first-line screening for haemoglobin variants and for HbA₂ quantitation:

- high performance liquid chromatography (HPLC), preferably with continuous gradient elution
- capillary electrophoresis (CE)

Both these techniques provide provisional results. Abnormal results must have further testing with a minimum of one alternate technique with a different analytical principle to the original and which is appropriate for the suspected variant (Table 1). **Minimum requirements for quality control material for these techniques can be found on GOV.UK^[1]**. In some cases more than one technique will be required. If results from all techniques are interpreted together laboratories can achieve a result which is sufficiently reliable for screening purposes. Only mass spectrometry and DNA provide a definitive diagnosis.

Table 1: Acceptable laboratory techniques.	
Initial method	Alternate method
HPLC	Alkaline electrophoresis
	Acid electrophoresis
	Isoelectric focusing (IEF)
	CE
	Mass spectrometry
	DNA
CE	Acid electrophoresis
	Alkaline electrophoresis ^a
	HPLC
	IEF
	Mass spectrometry
	DNA

^aResults from alkaline electrophoresis are very similar to CE, so this should not be used as the only alternate test. In the absence of IEF it may be useful in combination with acid electrophoresis.

In most circumstances sickle solubility testing can be used as confirmation of an initial screen that suggests the presence of HbS. There is an inherent unreliability in the sickle solubility test with the risk of false positive and negative results. Special care must be taken when using an instrument where HbS co-elutes with another haemoglobin variant, such as HbC. For example, in cases of co-eluting haemoglobin variants S and C, a negative solubility test cannot be presumed to indicate HbC and a positive solubility alone cannot be presumed to indicate HbS. In all cases where haemoglobins co-elute, it is necessary to use an additional technique, for example alkaline electrophoresis or IEF.

HbA₂ measurement

HPLC and CE methods are acceptable. IEF and scanning densitometry are not acceptable.

HbF measurement

HPLC, CE or 2 minute alkali denaturation are acceptable. The Kleihauer test is not appropriate for measurement but is useful to support the identification of HbF.

General laboratory considerations

Users must be aware that this laboratory handbook highlights common analytical and diagnostic issues but every laboratory must follow the principles of good laboratory practice. Laboratories must satisfy 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 external quality assurance (EQA). The manufacturer's published recommendations should be followed. Where an instrument operates with more than one analytical programme designed for haemoglobin variant analysis both programmes must be validated to ensure there are no significant differences where a numerical value is to be reported. If significant differences are found a single programme should be chosen based on the validation data, from which all results are reported.

It may be necessary to return to the original specimen tube 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.

For all analytical techniques, appropriate controls must be included wherever possible. If IEF or electrophoresis is used, then control haemoglobins must be run with each plate.

Minimum requirements for quality control material for these techniques can be found at GOV.UK^[1].

If a column is used this must be replaced once it has performed the recommended number of analyses as stated in writing by the manufacturer or if the performance is observed to deteriorate. If software rules are used to screen samples for further action and reporting, it is essential that the process is risk assessed and that there are failsafe mechanisms in place. Raw data, including analytical traces, must always be reviewed and any post analytical procedures, including algorithms, must be fully documented and traceable to ensure consistency of quality. All raw data, including analytical traces and interpretative comments, must be reviewed by 2 people, one of whom must be a registered scientist. Final validation must be by a person with suitable expertise, as demonstrated by current competency testing.

Selecting an analytical system

Co-ordination of pathology services across chemistry and haematology can allow the sharing of equipment, for example with that used for measurement of HbA_{1c}. In all situations, it is essential that HbA₂ is analysed by a protocol that calibrates and clearly separates HbA₂ from HbA. The required antenatal turnaround time of 3 working days for the issue of a report must be considered when this approach is used.

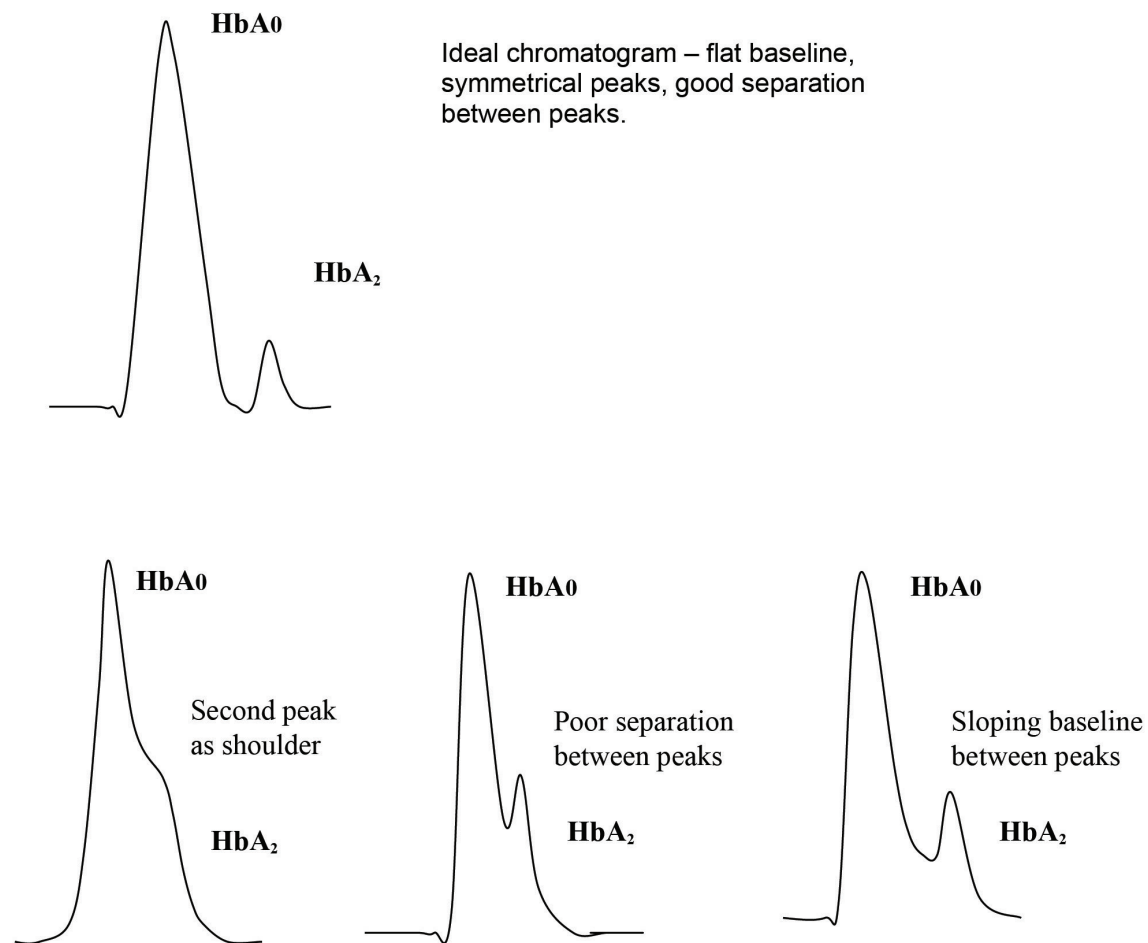
High performance liquid chromatography

HPLC uses an ion exchange resin, held in a column, in conjunction with a buffer gradient. As the ionic strength and/or pH of the buffer changes, 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. HbF and HbA₂ elute separately and the relative proportion is calculated with the use of calibrators. Ideally haemoglobins S, C, D, E and O^{Arab} should have separate retention times and characteristic chromatographic profiles. In addition, the relative proportions of the different haemoglobins are recorded. HPLC analysers that use step-wise buffer gradients are not recommended if they produce co-elution of common haemoglobin variants which can lead to misinterpretation.

When selecting an analyser for quantitation of HbA₂ the first consideration is good separation. It is important to ensure the analyser clearly separates peaks to ensure accurate quantitation as shown in Figure 1. Laboratories 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. Sophisticated integration and the use of calibration factors cannot make up for poor chromatography.

Figure 1. Examples of integration.



Capillary electrophoresis

CE uses 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, so position is not a unique identifier. HbF and HbA₂ elute separately and the relative proportion of each is calculated with the use of calibrators. Haemoglobins S, C, D, E and O^{Arab} also have separate retention times and characteristic profiles. In addition, the relative proportions of the different haemoglobins are recorded.

Optical density (OD) levels greater than 0.07 and the presence of sufficient HbA 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 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 may lead to misinterpretation of the results.

Action Values

A single value for HbA₂ of equal to or greater than 3.5% has been set as the action value for carriers of β thalassaemia. There are 2 action values for HbF. If the MCH is greater than or equal to 27pg, the action value is greater than 10%. However, if the MCH is less than 27pg, the action value is greater than or equal to 5.0%. The chosen system must be able to measure HbA₂ and HbF with accuracy and precision at these action values and detect the haemoglobin variants as specified by the antenatal screening programme. Instrument validation protocols must assess these requirements. Quantitation at different levels may be needed for other clinical purposes.

UK national external quality assurance scheme (UK NEQAS) data and published literature have demonstrated biases between different analysers for HbA₂. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the International Council for Standardization in Haematology (ICSH) have recognised the need for a new certified reference material for HbA₂, which is under development. The screening programme supports the objectives of this work and continues to work with UK NEQAS to monitor the impact of inter-method variability.

Laboratories in the UK should be aware of these problems and ensure they have optimised their methods. When considering replacement purchases, laboratories should review all available evidence, including UK NEQAS data.

Analytical systems should be able to detect and measure at least the most common HbA₂ variant (HbA₂Prime (A₂')) to enable an approximate calculation of total HbA₂ (for example HbA₂ plus A₂').

If using equipment or an elution programme for more than one analyte, for example HbA₂ and HbA1c, laboratories should ensure that the quantitation of HbA₂ and HbF is not compromised.

Good practice and troubleshooting

When operating analytical systems the following points of good practice should be followed and may be used as a troubleshooting guide if problems are identified by internal and/or external quality assurance.

1. Ensure the HPLC column count is within manufacturer's written guaranteed limit.
2. Make sure the calibration factor does not drift significantly or fall outside recommended limits.
3. Monitor internal quality control for drift.
4. Follow the manufacturer's recommended maintenance and cleaning protocols.
5. Ensure that reagents, controls and calibrators are stored in the correct conditions, are within date, have appropriate lot numbers and are in the correct positions.
6. Avoid pooling and mixing reagents, do not pool reagents with different lot numbers.
7. When reviewing analytical traces, ensure the appearance is as it should be with the correct peak shape, baseline and separation from adjacent haemoglobin peaks.
8. Ensure maintenance visits are carried out at the recommended intervals.
9. If the machine is moved or laboratory conditions change, appropriate revalidation procedures must be performed.
10. If problems occur that cannot be resolved contact the instrument manufacturer.

If samples require modification by the addition of extraneous haemoglobin an aliquot must be used. This must be analysed with a unique identifier distinguishable from both the original specimen and from any other clinical sample.

In the event of an out of consensus EQA result a look back exercise must be considered. Look back may also be required when internal quality assurance falls outside acceptable limits or when indicated for any other reason. Guidance is provided in Appendix 1.

Problems with the measurement and interpretation of HbA₂

In the presence of an α chain variant it can be difficult to obtain a reliable HbA₂ value. For this reason, where the biological mother has an MCH of less than 27pg and is found to have a suspected α chain variant or a haemoglobin variant that co-elutes with, or compromises the HbA₂, the baby's biological father must be offered screening. If the biological father has an MCH of less than 27pg with a suspected α chain variant or a variant which co-elutes with, or compromises the HbA₂ and the biological mother is known to have β thalassaemia, or has Hb S, E, O^{Arab} or Lepore and/or is at risk of alpha zero (α^0) thalassaemia, DNA studies should be performed.

If any small peaks which could be an HbA₂ variant elute separately from the main HbA₂ peak in a patient with an MCH below the action value (< 27pg), further investigation will be required if the addition of these peaks brings the total HbA₂ greater than or equal to 3.5%.

HbA₂ values of 4.0% and above with normal indices may indicate a carrier of β thalassaemia. In this case the findings including the FBC should be confirmed. If the results remain the same then the baby's biological father must be tested. Other factors such as HIV infection, B12/folate deficiency or liver disease/alcohol can increase the HbA₂.

HbA₂ values less than 4.0% with normal red cell indices and an HbF level of less than or equal to 10% are regarded as not significant for screening purposes.

With many HPLC systems, HbA₂ is overestimated in the presence of HbS. This is not a problem where the percentage of HbA is greater than HbS. See section 'interpretation of relative proportions' on [page 31](#).

With many HPLC systems, HbA₂ is underestimated in the presence of HbD. This is not a problem where the percentage of HbA is greater than HbD but may make discrimination between Hb DD and D/ β^0 thalassaemia more difficult. Ensure red cell indices are reviewed.

When using CE, HbA₂ is underestimated in the presence of HbC. This is not a problem where the percentage of HbA is greater than HbC but may make discrimination between Hb CC and C/β⁰ thalassaemia more difficult. Ensure red cell indices are reviewed.

For analysers that separate and measure HbA₂ in the presence of HbE, caution should be used when interpreting this value.

Iron deficiency

The HbA₂ level may be lowered by up to 0.5% in cases of severe iron deficiency anaemia^[6] but 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 HbA₂ should be measured. It may be appropriate to simultaneously investigate pregnant women for iron deficiency, using ferritin or zinc protoporphyrin but this is not specifically part of the screening protocols.

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

Screening for carriers of α⁰ thalassaemia

Methods of screening

The lack of a specific biomarker for the detection of α thalassaemia carriers creates problems, particularly in the context of a screening programme. In this context α⁺ thalassaemia is not regarded as significant and policies are designed to detect only couples at risk of hydrops fetalis. Policy has been designed to increase the positive predictive value of the screening algorithm and reduce false positives.

Diagnosis

Molecular techniques must be used for the confirmation and diagnosis of α⁰ thalassaemia when suspected in both biological parents. α thalassaemia mutations may be both deletional and non-deletional, requiring the use of different diagnostic techniques. For the common deletional mutations laboratories using GAP PCR based techniques will only detect those mutations included in their screening panels.

Population estimates and ethnic distribution of α^0 thalassaemia

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

Alpha zero thalassaemia is most commonly found in those of Southeast Asian origin (China including Hong Kong, Thailand, Taiwan, Cambodia, Laos, Vietnam, Indonesia, Burma, Malaysia, Singapore or Philippines) and East Mediterranean (Cyprus, Greece, Sardinia or Turkey). There are 2 common Mediterranean (--MED, $-\alpha(20.5)$) and 3 common Southeast Asian deletional mutations (--SEA, --THAI, --FIL).

Alpha zero 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 $-\alpha(20.5)$ allele in Iran; the --YEM allele in the Yemen. α^0 thalassaemia is rarely reported in patients of African, Pakistani and Indian origin. α^0 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 high risk for α^0 thalassaemia^[7].

Further investigation of α^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 α thalassaemia major is at risk of obstetric complications, such as toxoemia and hypertension, particularly in the third trimester of pregnancy. If a fetus with α thalassaemia major is transfused *in utero*, it is possible that it will survive and be born as a transfusion-dependent infant. Usually a baby only has Hb Bart's hydrops fetalis (α thalassaemia major) if both biological parents are carriers of α^0 thalassaemia.

Table 2 shows the α thalassaemia genotypes.

Genotype	Phenotype
$\alpha\alpha/\alpha\alpha$	Normal
$-\alpha/\alpha\alpha$	Alpha plus (α^+) thalassaemia (heterozygote)
$-\alpha/-\alpha$	Alpha plus (α^+) thalassaemia (homozygote)
$--/\alpha\alpha$	Alpha zero (α^0) thalassaemia (heterozygote)
$--/-\alpha$	Haemoglobin H disease
$--/--$	Alpha thalassaemia major (homozygote) Hb Bart's hydrops fetalis

The approach to screening and α^0 thalassaemia

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

1. Is the MCH <25pg?
2. Is the woman's family origin identified as high risk from the [FOQ](#): 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 biological father must be offered if he is also from a high risk area or unknown.

If the baby's biological father is suspected of having α^0 thalassaemia, the samples on both biological parents must be sent for DNA analysis for α^0 thalassaemia mutations.

If one biological parent is a suspected carrier of α^0 thalassaemia and the other is a carrier of α thalassaemia and is also from one of the high risk groups for α^0 thalassaemia with an MCH < 25pg, both biological parents should be screened for α^0 thalassaemia by DNA analysis.

DNA analysis of samples and α^0 thalassaemia

The policy guidance developed by the screening programme should mean that DNA analysis is only done in a limited number of cases and not usually in cases where α^+ thalassaemia is suspected.

Evidence for above approach

Published studies have shown that 99% of α^0 thalassaemia cases have an MCH <25pg. In a series of 270 carriers of α^0 thalassaemia diagnosed by DNA analysis in the UK, only 2 patients had an MCH between 25 and 26pg – one with liver disease^[8]. Findings in a study from Sheffield^[9] which undertook DNA analysis in 425 pregnant women with an MCH <27pg showed that all cases of α^0 thalassaemia had an MCH <25pg and would have been detected using an [FOQ](#) alone, which supports the screening programme's approach.

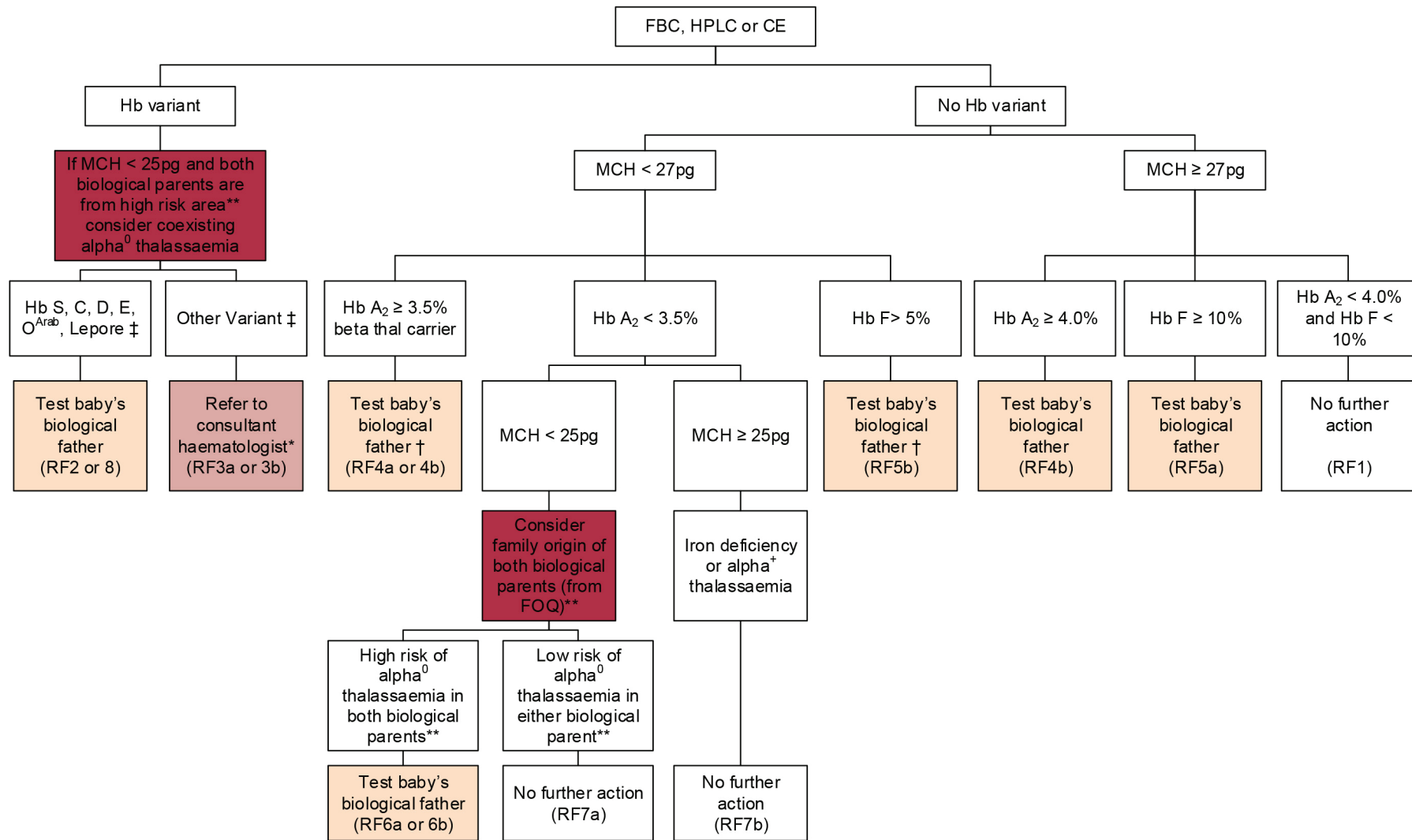
Risk assessment for antenatal screening in high prevalence areas

It is inevitable there will be some false positives and negatives as a result of screening because of the way screening programmes are designed, particularly with the use of action values. False positives have a positive result from the screening test but, when further tests have been performed, do not have one of the designated haemoglobins or thalassaemia. Potential causes of false negatives are shown below.

Conditions that may be missed using the algorithm (assuming that the [FOQ](#) has been completed accurately) include:

- 'silent' or 'near silent' β thalassaemia carriers. Some β thalassaemia carrier genotypes are associated with borderline HbA₂ levels and an action value of 3.5% with an MCH of < 27pg will miss some cases. Examples of such mutations include the c.-50 A>C [CAP+1 (A>C)], c.92+6 T>C [IVSI-6 (T>C)], c.-151C>T [-101 (C>T)] and c.*111 A>G or c.*110 T>C [Poly A (A>G) or (T>C)]
- some β thalassaemia carriers obscured by severe iron deficiency anaemia
- thalassaemia where the MCH is raised, for example B12/folate deficiency, liver disease, HIV therapy
- β thalassaemia carriers with a co-existing δ chain mutation which is silent with the first line screening technique, or who have co-existing delta thalassaemia
- β thalassaemia carriers with co-existing HbH Disease as some cases have normal HbA₂ values
- α^0 thalassaemia occurring outside the defined at risk family origins (see [page 18](#)) or in those women with an MCH ≥ 25 pg
- $\delta\beta$ thalassaemia carriers with HbF $\leq 5\%$
- $\gamma\delta\beta$ thalassaemia carriers
- dominant haemoglobinopathies in the baby's biological father when the woman is Hb AA, these are very rare and should be suggested by the family history
- any significant condition silent with the first line screening
- any significant condition masked by a blood transfusion
- any significant condition masked by an unreported bone marrow transplant
- any significant condition present in donor egg or sperm where the donor is undeclared or untested
- incorrect family origins assigned due to undeclared adoption

Figure 2 - Testing algorithm for laboratory screening in high prevalence areas.



* 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 or unknown.

Reconsider low risk couples if fetal anaemia/hydrops seen on ultrasound scanning or if family history of hydrops fetalis.

† In all cases consider coexisting α^0 thalassaemia if both parents are from a high risk area and MCH < 25pg.

‡ Consider coexisting beta thalassaemia

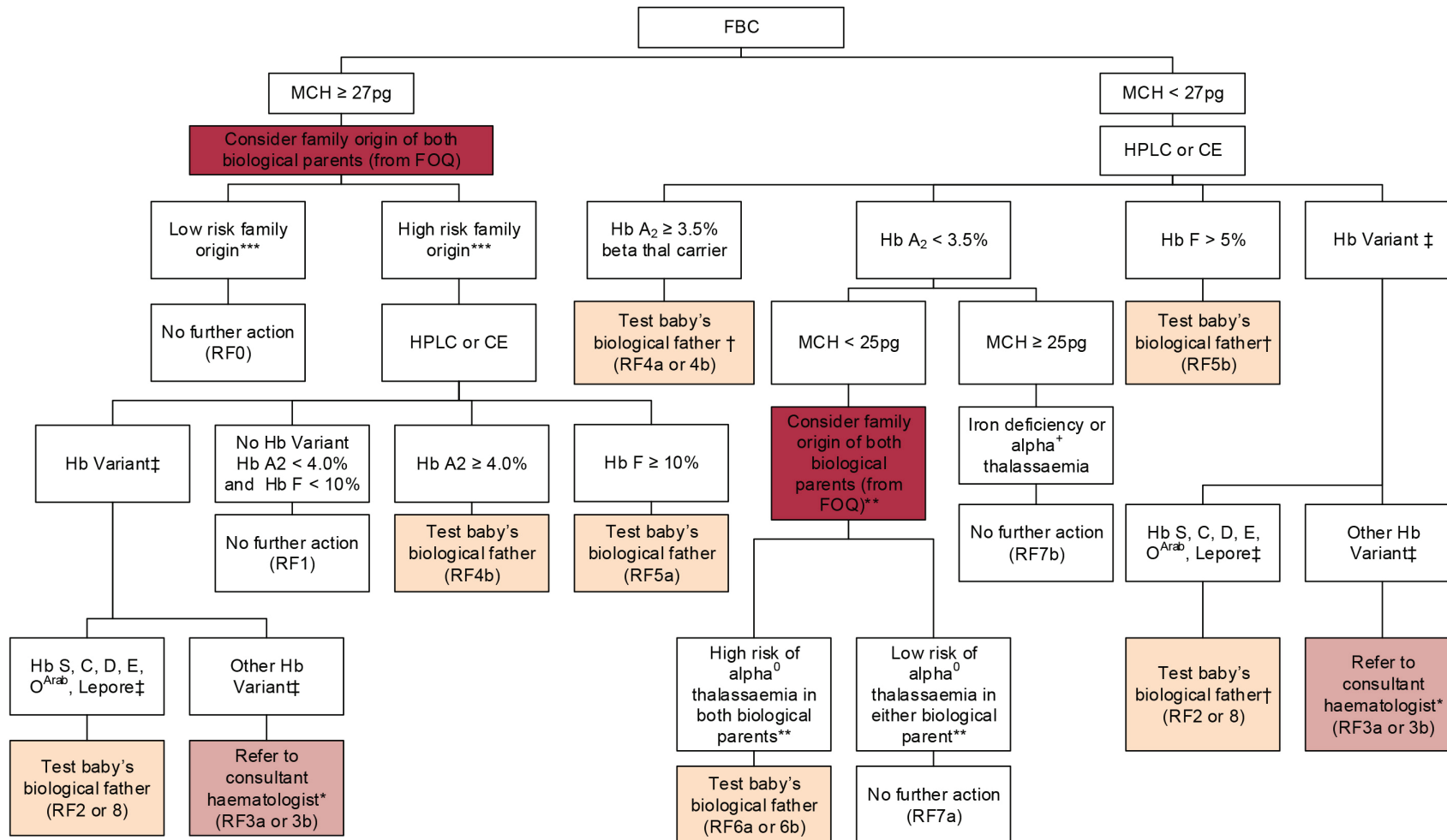
Risk assessment for antenatal screening in low prevalence areas

It is inevitable there will be some false positives and negatives as a result of screening because of the way screening programmes are designed, particularly with the use of action values. False positives have a positive result from the screening test but, when further tests have been performed, do not have one of the designated haemoglobins or thalassaemia. Potential causes of false negatives are shown below.

Conditions that may be missed using the algorithm (assuming that the FOO has been completed accurately) include:

- 'silent' or 'near silent' β thalassaemia carriers. Some β thalassaemia carrier genotypes are associated with borderline HbA₂ 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 c.-50 A>C [CAP+1 (A>C)], c.92+6 T>C [IVSI-6 (T>C)], c.-151C>T [-101 (C>T)] and c.*111 A>G or c.*110 T>C [Poly A (A>G) or (T>C)].
- some β thalassaemia carriers obscured by severe iron deficiency anaemia
- thalassaemia where the MCH is raised eg B12/folate deficiency, liver disease, HIV therapy
- β 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.
- β thalassaemia carriers with co-existing HbH Disease as some cases have normal HbA₂ values.
- a⁰ thalassaemia occurring outside the defined at risk family origins (see [page 18](#)) or in those women with an MCH \geq 25pg.
- $\delta\beta$ thalassaemia carriers with HbF \leq 5%
- $\gamma\delta\beta$ thalassaemia carriers
- dominant haemoglobinopathies in the baby's biological father when the woman is Hb AA, these are very rare and should be suggested by the family history
- Hb S, C, D^{Punjab}, E, O^{Arab} outside the defined at risk family origins
- any significant condition silent with the first line variant screening technique
- any significant condition masked by a blood transfusion
- any significant condition masked by an unreported bone marrow transplant
- any significant condition present in donor egg or sperm where the donor is undeclared or untested
- incorrect family origins assigned due to undeclared adoption

Figure 3 - Testing algorithm for laboratory screening in low prevalence areas.



* 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 or unknown.
 *** Low risk or high risk as determined by the family origin questionnaire. **Note: If baby's father is in high risk group, test the mother's sample regardless of her family origins.** Reconsider low risk couples if fetal anaemia/hydrops seen on ultrasound scanning or if family history of hydrops fetalis.
 † In all cases consider coexisting α⁰ thalassaemia if both parents are from a high risk area and MCH <25pg.
 ‡ Consider co-existing beta thalassaemia

Interpretation and reporting of antenatal screening results

These guidelines should provide sufficient information to allow all normal, and over 95% of abnormal, cases to be reported in a standardised manner. There will always be some situations that require further tests, or family studies, before a useful clinical result can be achieved because of the diversity of haemoglobin variants and thalassaemia syndromes. Appropriate clinical referral is required in these cases to ensure that the risk to the pregnancy is fully and correctly assessed.

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 essential that clinicians realise this fact and it is best practice to have a universal footnote on all haemoglobinopathy results, such as: 'unless otherwise stated, result assumes no recent transfusion' or 'result valid if not transfused'.

Laboratories should treat haemoglobin values of less than 80g/L with caution. There are multiple conditions that can cause severe anaemia in pregnancy, including iron deficiency, haemolytic anaemia, HbH disease, or β thalassaemia intermedia. Laboratories should review these on a case-by-case basis. If necessary, they should discuss such cases with a senior scientist or clinician to consider appropriate further action, including testing of the baby's biological father.

It is not possible to separate all haemoglobin variants using screening methods and a second haemoglobin variant may be migrating/eluting with any other haemoglobin including HbA. This is a particular issue in HPLC instruments which use a stepwise buffer gradient, notably the co-elution of Hbs S and C. Conversely such gradients may also split other haemoglobin variants into 2 peaks, for example HbD^{Punjab}. In other cases, such as when thalassaemia and haemoglobin variants are co-inherited, one condition may be masking another.

HPLC instruments separate adducted fractions of haemoglobins, these fractions must not be added to the assumed parent peak. Addition of assumed peaks can lead to erroneous results. No additional testing is necessary if the HbA₂ is raised (but not greater than 8%) and the red cell indices are typical of a carrier of β thalassaemia. It is important to be aware that HbF levels are highly variable in β thalassaemia carriers.

Screening shows the presence of HbA only: possible scenarios

Scenario 1

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is ≥ 27 pg
- HbA₂ is $< 4.0\%$
- HbF is $< 10\%$
- Hb is ≥ 80 g/L

Then no further testing is required. There is negligible risk in the pregnancy associated with an abnormal haemoglobin or thalassaemia. **Use report format 1**

Scenario 2

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is < 27 pg
- HbA₂ is $\geq 4.0\%$ but $< 8.0\%$

Then there is a risk in the pregnancy associated with the presence of β thalassaemia. Use report format 4a. If the HbA₂ is $> 8.0\%$ check for other variants, particularly Hb Lepore.

Scenario 3

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is ≥ 27 pg
- HbA₂ is $\geq 4.0\%$ but $< 8.0\%$

Then there is a risk in the pregnancy associated with the presence of β thalassaemia. Use report format 4b. If the HbA₂ $> 8.0\%$, check for other variants using an alternate technique.

Scenario 4

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is $< 27\text{pg}$
- HbA₂ is $\geq 3.5\%$ but $< 4.0\%$

Then there is a risk in the pregnancy associated with the presence of β thalassaemia. Use report format 4b.

Scenario 5

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is $< 27\text{pg}$
- Hb is $< 80\text{g/L}$
- HbA₂ is $\geq 3.0\%$ but $< 4.0\%$

Then there is a risk in the pregnancy associated with the presence of β thalassaemia. Use report format 4b.

Scenario 6

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is $< 27\text{pg}$
- HbA₂ is $< 3.5\%$

Consider whether this is the measurement of the total HbA₂. If the patient has a delta chain variant or an α chain variant, a second HbA₂ peak/band may be present and must be included in the total HbA₂ where appropriate. If total HbA₂ (HbA₂ plus HbA₂ variant) $\geq 3.5\%$ then there may be a risk in the pregnancy associated with the presence of β thalassaemia. **If β thalassaemia is suspected use report format 4a or 4b as described in scenarios 3 to 5.**

If the HbA₂ measurement is correct, $< 3.5\%$ please see scenarios 7 to 9. In these scenarios the [FOQ](#) forms part of the screening tests.

Scenario 7

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is $< 27\text{pg}$ but $\geq 25\text{pg}$
- HbA₂ is $< 3.5\%$
- HbF is $< 5.0\%$
- Hb is $\geq 80\text{g/L}$

This indicates possible iron deficiency or α^+ thalassaemia. There is negligible risk to the pregnancy. **Use report format 7b.**

Scenario 8

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is $< 25\text{pg}$
- HbA₂ is $< 3.5\%$
- HbF is $< 5.0\%$
- biological parents are both of high risk family origins for α^0 thalassaemia or unknown or one has family origins of high risk and the other is unknown

This indicates possible α^0 thalassaemia or iron deficiency. Use report format 6a. If the woman has HbH disease, use report format 6b.

Scenario 9

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is < 25pg
- HbA₂ is < 3.5%
- HbF is < 5.0%
- **neither, or only one, biological parent has family origins of high risk for α^0 thalassaemia**

This indicates possible iron deficiency, homozygous α^+ thalassaemia, or heterozygous α^0 thalassaemia (**use report format 7a**) or HbH disease (**use report format 6b**).

Scenario 10

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is ≥ 27 pg
- HbA₂ is < 4.0%
- HbF is $\geq 10\%$
- Hb is ≥ 80 g/L

There is a risk to the pregnancy associated with the presence of HPFH. Use report format 5a. **If Hb is < 80g/L ensure that all other potential risks have been considered.**

Scenario 11

If screening tests show only a single major Hb peak (band) in the HbA position and the:

- MCH is < 27pg
- HbA₂ is < 3.5%
- HbF is ≥ 5.0%

There is a risk to the pregnancy associated with the presence of $\delta\beta$ thalassaemia. Use report format 5b

The possible scenarios are summarised in Table 3.

Table 3 needs to be read in conjunction with 'interpretation and reporting of antenatal screening results' on [page 23](#) and recommended report formats [page 34](#).

Table 3. Summary of interpretation and reporting guidelines. [Where screening shows a single peak in the position of HbA.]

Family origin	MCH pg	Total A2 %	F %	Hb (g/L)	Conclusion	Report format
Any	≥ 27	< 4.0	< 10	≥ 80	No evidence of abnormal haemoglobin or thalassaemia	1
Any	≥ 27	≥ 4 but < 8.0	—	—	Possible β thalassaemia carrier	4b
Any	≥ 27	≥ 8.0	—	—	Check for variant haemoglobins	—
Any	< 27	≥ 4 but < 8.0	—	—	β thalassaemia carrier ^a	4a
Any	< 27	≥ 8.0	—	—	Check for variant haemoglobins, particularly Hb Lepore ^a	—
Any	< 27	≥ 3.5 but < 4.0	—	—	Possible β thalassaemia carrier ^a	4b
Any	< 27	> 3.0 but < 4.0	—	< 80	Possible β thalassaemia carrier ^a	4b
Any	< 27	< 3.5	—	—	Check for a second HbA ₂ , due to either an α or a δ chain variant ^a	—
Any	< 27pg but ≥ 25pg	< 3.5	—	≥ 80	Possible iron deficiency or α thalassaemia	7b
Both biological parents high risk for α ⁰ thalassaemia or unknown or one high risk and one unknown	< 25	< 3.5	—	≥ 80 ^b	Possible α ⁰ thalassaemia or iron deficiency or HbH Disease. Baby's biological father requires testing.	6a or 6b
Neither, or only one, biological parent has family origins of high risk for α ⁰ thalassaemia	< 25	< 3.5	< 5	≥ 80 ^b	Possible iron deficiency or homozygous α ⁺ thalassaemia or heterozygous α ⁰ thalassaemia or HbH Disease. Testing of baby's biological father not required.	7a or 6b
Any	≥ 27	< 4	< 5	≥ 80	Hereditary persistence of fetal haemoglobin	5a
Any	< 27	< 3.5	< 5	—	δβthalassaemia carrier ^a	5b

^aConsider coexisting α⁰ thalassaemia when MCH < 25pg and the biological parents have appropriate family origins (see algorithm)

^bUnless proven HbH Disease where Hb may be lower

First test suggests HbA and either HbS, HbC, HbD, HbE or HbO^{Arab}

Further tests required:

Sickle solubility test

It is best practice to perform a sickle solubility on all haemoglobin variants detected. If the variant has features suggestive of HbS and the sickle solubility is positive no further testing is usually required. However, see note on [page 10](#) about the need for caution when using the sickle solubility test for confirmatory purposes with certain instruments. If a sickling haemoglobin is present at < 20% the sickle test may not be positive and in this case further investigations will be required.

For all other variants see Table 4

Table 4 gives the minimum testing requirements to support the presumed identity of the listed haemoglobin variants when using HPLC or CE as the first test. Laboratories may choose to perform additional or more specific techniques but must use a method with a different scientific principle to the primary method (Table 1). Minimum requirements for quality control material for these techniques can be found at GOV.UK^[1]

Table 4. Minimum testing requirements.	
Suspected variant	Additional techniques
HbC	Either acid electrophoresis or IEF
HbD	^a Either alkaline electrophoresis or IEF and acid electrophoresis
HbE	Either alkaline electrophoresis or IEF and acid electrophoresis
HbO ^{Arab}	Acid electrophoresis
Hb Lepore	Either alkaline electrophoresis or IEF and acid electrophoresis

^aThere are several D haemoglobins, but the screening programme aims to detect HbD^{Punjab}. When HbD is detected and it cannot be differentiated, testing of the baby’s biological father will identify the couples at potential risk. The HbD will need to be confirmed as HbD^{Punjab} if the baby’s biological father is found to have HbS. This will also apply if the biological mother has HbS and the baby’s biological father is found to have HbD. In such cases confirmatory testing must be either DNA or mass spectrometry.

Interpretation of relative proportions

Hb Variant (V) between 40 and 50% (35 and 50% if HbV is HbE). Is the band/peak in the HbA position actually HbA? If the HbV > HbA the possibility exists of Hb V/ β^+ thalassaemia with an unusually high expression of HbA. Unless β thalassaemia is also present the antenatal risk is only for HbV.

HbV > 50% – consider Hb V/ β^+ thalassaemia. Cases of Hb V/ β^+ thalassaemia will usually have a reduced MCH. Consider the HbA₂, although the presence of a haemoglobin variant may affect the values obtained on many systems. Ensure that red cell indices are reviewed and where V/ β^+ thalassaemia is suspected further investigation is required. In the case of V/ β^+ thalassaemia the antenatal risk is for both HbV and β thalassaemia.

HbV < 30% (< 25% if HbV is HbE) and MCH < 25pg – HbV carrier with possible co-existing α thalassaemia or iron deficiency. The α thalassaemia risk needs to be considered in the light of the family origin of both biological parents – see algorithm for α thalassaemia. The major risk is for HbV, but the risk of hydrops fetalis must not be overlooked especially if HbV is HbE.

In all the above cases use report format 2 or 8 as appropriate.

Variant specific considerations

Refer to section 'Problems with the measurement and interpretation of HbA₂', [page 15](#).

HbS

Be aware that with some HPLC systems adducts of HbS may elute in the HbA window and may be mistaken for a small amount of HbA, so a person with sickle cell disease may appear to have Hb S/ β^+ thalassaemia. If there is doubt as to the identity of this small peak an alternate method to confirm or refute the presence of HbA must be used.

HbC

The position of HbC relative to other haemoglobins can vary considerably depending upon the system used. On some HPLC systems, HbC gives a small additional peak of adducts of HbC in the HbS window.

HbD

The position of HbD will depend upon the system in use. Some systems underestimate the HbA₂ level in the presence of HbD^{Punjab}. This should not cause a diagnostic problem except when differentiating between Hb D/β⁰ thalassaemia and Hb DD when it is essential to review the red cell indices.

HbE

With some HPLC methods, HbE appears as a peak in the HbA₂ window, so the levels of both HbE and HbA₂ cannot be estimated accurately. The percentages given above can vary depending on the analyser in use. The HbE mutation creates an alternate splicing site in the messenger RNA resulting in reduced expression. This, together with the instability of HbE, means there is less of the variant when compared with other β chain mutations phenotypically. There is often associated microcytosis and slightly raised HbA₂. Where they are measured separately the reference range for HbA₂ is unlikely to apply.

HbO^{Arab}

HbO^{Arab} appears in different positions on different analysers but frequently appears in close proximity to HbC.

Hb Lepore

Hb Lepore appears in different positions depending on the analyser used. Quite often it appears as an oddly shaped elevated HbA₂ peak with values up to 15% and therefore the levels of neither Hb Lepore nor HbA₂ can be estimated accurately. By IEF there is a band in HbG^{Philadelphia} position and by alkaline electrophoresis, a band just faster than HbS, but this may be difficult to separate from HbS. The MCH is usually < 27pg.

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

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

Homozygotes and compound heterozygotes

Where a single major peak (band) is detected, co-existing β thalassaemia must always be considered. The red cell indices and HbA₂ level need to be reviewed. Remember to allow for the variable nature of HbA₂ levels in the presence of Hb

variants. Refer to section 'Problems with the measurement and interpretation of HbA₂', [page 15](#). Once coexisting β thalassaemia has been excluded, the presence of single peak/band is most likely due to homozygosity for a Hb variant. Compound heterozygotes for haemoglobin variants usually have 2 peaks/bands equating to the 2 haemoglobin variants present, but it is possible for these to co-migrate.

The α thalassaemia risk needs to be considered in the light of the family origin of both biological parents – see algorithm for α thalassaemia.

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

Reporting antenatal screening results

All reports must include a sample date. The FBC must be reviewed and included in the haemoglobinopathy report as it may be the only indication of thalassaemia. Where decisions are based on information derived from the [FOQ](#) or from FBC parameters from an alternate source, for example from a referring laboratory, it is best practice to include that information on the report. If you are reporting the standard abbreviations, for example HbAS, you must follow the convention for the haemoglobins present to be reported in the order of greatest to least percentage. You must separate analytical fact from interpretative opinion. The factual results should be given and then a clear conclusion given in full text, which must include recommendations. If a final result cannot be produced within the 3 working day standard for reporting, an interim report, sufficient for the woman's clinical care and recommending the testing of the baby's biological father if necessary, must be issued.

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 essential clinicians realise this fact and it is best practice to have a universal footnote on all haemoglobinopathy results such as: 'unless otherwise stated, result assumes no recent transfusion' or 'result valid if not transfused'.

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
- thalassaemia carriers
- homozygote and compound heterozygote conditions

The form of words in the report may differ depending on the local protocols of the screening laboratory. It is possible that the comment about testing the baby's biological father will not be needed in all laboratories if alternative protocols are used by the screening service to initiate such requests. Comments highlighted in **bold** are intended as prompts for the laboratory and should not be reported verbatim.

Care should be taken when reporting coexisting conditions to ensure that all risks to the baby are considered when the results of both biological parents are reviewed.

Report format 0: for specimens screened by red cell indices only (low prevalence areas).

Report the red cell indices with the comments:

No evidence of thalassaemia.

Not tested for haemoglobin variants as family origin questionnaire indicates both biological parents are of low risk family origins.

Testing of baby's biological father not required.

Report format 1: no abnormality detected.

Report the red cell indices and other results as appropriate together with the comments:

No evidence of an abnormal haemoglobin or thalassaemia. Testing of baby's biological father not required.

Report format 2: for the following haemoglobin variant carriers: HbS, HbC, HbD, HbE, HbO^{Arab} and Hb Lepore.

Report the red cell indices, sickle cell solubility test and other results as appropriate together with the comments:

Results consistent with Hb'V' carrier (Hb AV) **where V is the variant haemoglobin detected.**

Where the variant is HbS the term 'sickle cell carrier' is preferred. When indicated comment on the possibility of coexisting α thalassaemia. Consider the possibility of α^0 thalassaemia when MCH < 25pg and there are high risk family origins for α^0 .

Testing of baby's biological father recommended.

Report format 3a: haemoglobin variant carriers where testing of the baby's biological father is not required.

Report the red cell indices, sickle cell solubility test and the other results as appropriate together with the comments:

Results consistent with Hb'V' carrier (Hb AV) of no known clinical significance.

Where V is the variant haemoglobin detected.

Testing of baby's biological father not required.

If α^0 thalassaemia is suspected (MCH < 25pg) then this must be reported appropriately. If family origin questionnaire indicates both biological parents are of high risk family origins for α^0 thalassaemia then the conclusion must be changed to recommend testing of baby's biological father.

Report format 3b: haemoglobin variant carriers where testing of the baby's biological father is required.

Report the red cell indices, sickle cell solubility test and the other results as appropriate together with the comments:

Results consistent with Hb'V' carrier (Hb AV) - **where V is the variant haemoglobin detected. This must be used for variants of clinical significance (not covered in report format 2) or variants of unknown clinical significance. If the variant is unidentified recommended wording is carrier of unidentified variant. When indicated comment on the possibility of coexisting α thalassaemia. Consider the possibility of α^0 thalassaemia when MCH < 25pg and there are high risk family origins for α^0 .**

Testing of baby's biological father recommended.

Report format 4a: for β thalassaemia carriers.

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with β thalassaemia carrier.

Comment on possibility of coexisting α^0 thalassaemia when MCH < 25pg and there are high risk family origins for α^0 .

Testing of baby's biological father recommended.

Report format 4b: for possible β thalassaemia carriers.

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with possible β thalassaemia carrier.

Comment on possibility of coexisting α^0 thalassaemia when MCH < 25pg and there are high risk family origins for α^0 .

Testing of baby's biological father recommended.

Report format 5a: for HPFH.

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with carrier of Hereditary Persistence of Fetal Haemoglobin.

Testing of baby's biological father recommended.

Report format 5b: for $\delta\beta$ thalassaemia carrier.

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with δ/β thalassaemia carrier.

Comment on the possibility of coexisting α^0 thalassaemia if the MCH < 25pg and there are high risk family origins for α^0 .

Testing of baby's biological father recommended.

Report format 6a – for possible α^0 thalassaemia carriers when both the biological mother and the baby's biological father are of high risk family origins for α^0 .

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with possible α thalassaemia carrier and/or iron deficiency.

Family origin questionnaire indicates both biological parents are of high risk family origins for α^0 .

Testing of baby's biological father recommended.

Report format 6b: for HbH disease

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with HbH disease.

Testing of baby's biological father recommended if he is of high risk family origins for α^0 thalassaemia.

Report format 7a: for possible α thalassaemia carriers when either biological parent is not of high risk α^0 thalassaemia family origins (MCH < 25pg)

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with possible α thalassaemia carrier and/or iron deficiency.

Testing of baby's biological father not required as one or both biological parents are of low risk family origins for α^0 thalassaemia.

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

Report the red cell indices and the other results as appropriate together with the comments:

Results consistent with possible iron deficiency and/or α thalassaemia carrier.

Testing of baby's biological father not required.

Report format 8: for homozygote and compound heterozygote conditions

Report the red cell indices, sickle cell solubility test and the other results as appropriate together with the comments:

Results consistent with:

- Sickle cell anaemia (Hb SS)
- Hb SC disease
- Hb SD disease
- Hb SE disease
- Hb SO^{Arab} disease
- Hb S/Lepore
- Sickle/ β thalassaemia
- Hb variant/ β thalassaemia

Or adapt this format appropriately to fit the haemoglobins present.

Potential clinically significant conditions must be referred to a Consultant Haematologist if not already in follow up.

Testing of baby's biological father recommended.

Comment on the risk of coexisting α^0 thalassaemia if the MCH <25pg and there are high risk family origins for α^0 .

Guidance on referral of samples for DNA

Table 5 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 an expert opinion.

Table 5. Referral guidelines for antenatal screening specimens.

Biological mother carrier state	Biological father carrier state	Further studies by DNA analysis
No abnormalities detected	Testing of baby's biological father not required	None required
Any abnormal Hb or thalassaemia	No abnormality detected	None required
HbS	HbS or HbC	None required until PND
HbS	HbO ^{Arab} , D ^{Punjab} , E, Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation
HbS	HPFH	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
HbS + α thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of α^0 thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of α^0 thalassaemia
HbC	HbS	None required until PND
HbD	HbS	If PND is being considered send bloods for mutation confirmation
HbO ^{Arab}	HbS, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation
Hb Lepore	HbS, E, O ^{Arab} , Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation
HbE	β thalassaemia, Hb Lepore, $\delta\beta$ thalassaemia, HbS	If PND is being considered send bloods for mutation confirmation
HbE	a thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia
β or $\delta\beta$ thalassaemia	HbS, E, O ^{Arab} , Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation
β or $\delta\beta$ thalassaemia	a thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia
HPFH	HbS, E, O ^{Arab} , Lepore, β or $\delta\beta$ thalassaemia	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
α thalassaemia (MCH < 27pg but \geq 25pg)	Testing of baby's biological father not required	None required
α thalassaemia (MCH < 25pg) 1) Low risk α^0 thal family origins in either biological parent 2) High risk α^0 thal family origins in both biological parents or unknown	Testing of baby's biological father not required Test baby's biological father and if MCH < 25pg irrespective of any other phenotype detected	None required Send maternal and paternal bloods for mutation confirmation

Quality assurance and accreditation

All commissioners and service providers must refer to the public health functions agreement (Section 7A) service specification (No 18), and supporting standards and handbook to ensure a service is set up correctly and meets the standards set by the national screening programme.

Laboratories offering screening for the NHS Sickle Cell Disease and Thalassaemia Screening Programme must:

- be accredited by the UK Accreditation Service (UKAS) to ISO. 'Medical laboratories – Requirements for quality and competence (ISO 15189) or be CPA accredited and actively transitioning towards ISO 15189
- participate in EQA schemes accredited to ISO. 'Conformity assessment. General requirements for proficiency testing schemes (ISO 17043)'
- meet the screening programme quality assurance requirements mapped to ISO 15189
- use ISO 15189 accredited reference laboratories

The UK Accreditation Service (UKAS) will assess both the ISO and the screening requirements on behalf of PHE Screening Quality Assurance Services and the NHS Sickle Cell and Thalassaemia Screening Programme.

Referral of antenatal samples for mutation analysis

The majority of couples at risk of having a child affected with β thalassaemia or sickle cell disease are identified initially through the antenatal screening programme. The diagnosis of α thalassaemia is more complicated because DNA analysis is the only way to distinguish between α^+ ($-\alpha/\alpha\alpha$), homozygous α^+ ($-\alpha/-\alpha$) and α^0 ($--/\alpha\alpha$) thalassaemia. Due to the high frequency of α^+ thalassaemia in some populations and the low cost benefit ratio in a screening programme, DNA confirmation is not required for all possible cases of α thalassaemia. The screening programme relies on a strategy for diagnosis of α^0 thalassaemia by combining haematological tests and details of family origin.

Although the antenatal screening programme guidelines are designed to identify most carriers for serious haemoglobinopathies, including thalassaemias, the screening protocols will not identify every couple at risk for every haemoglobinopathy. For example, they are not designed to pick up couples at risk for HbH disease or the extremely rare cases of couples of low risk family origins on the [FOQ](#) who are at risk for Hb Bart's hydrops fetalis syndrome.

Testing of the baby's biological father

This must be performed according to screening protocols as for maternal phenotype testing. If the baby's biological father has a phenotype that can interact with the maternal phenotype as depicted in Table 5, then the couple should be counselled. If PND is being considered, then blood samples on both biological parents must be sent to a DNA referral laboratory.

If the baby's biological 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 PND in this situation. PND can be undertaken without the DNA of the baby's biological father, although in some circumstances the diagnosis will not be able to be given with such a high degree of certainty as when the baby's biological father's mutation is known.

Guidelines on the referral of blood samples for DNA analysis

The blood samples must be accompanied by an appropriately completed DNA laboratory referral form and the laboratory notified prior to sending samples.

Haemoglobin variants

If both biological parents carry HbS, or one carries S and one carries C then:

- blood samples are not required immediately to confirm the mutation by DNA analysis

If PND has been accepted, fresh blood samples from both biological parents should be sent at the same time as the fetal sample.

If one biological parent carries HbS and the other is a suspected carrier of HbO^{Arab}, HbD^{Punjab}, HbE, Hb Lepore or a type of β thalassaemia (including $\delta\beta$ thalassaemia) then:

- if time permits blood samples from both biological parents should be sent to confirm the mutations prior to fetal sampling
- if PND has been accepted, a fresh maternal sample (and paternal sample) if not previously tested as above should be sent at the same time as the fetal sample

If one biological parent carries HbS and the other is thought to carry deletional HPFH then:

- PND is not usually indicated but it is important to ensure that deletional HPFH is differentiated from $\delta\beta$ thalassaemia (these cases may require mutational analysis)

Carriers of deletional HPFH have a raised HbF level of approximately 20 to 30% and 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 HbF level usually in the range of 5 to 15%. Non-deletional HPFH usually results in a more modest increase of HbF (less than 10%) in adults and is found in many populations. It is usually associated with normal red cell indices.

If one biological parent carries HbE and the other carries β thalassaemia, Hb Lepore, $\delta\beta$ thalassaemia or is thought to carry α^0 thalassaemia then:

- if time permits blood samples from both biological parents should be sent to confirm the mutations prior to fetal sampling
- if PND has been accepted, a fresh maternal sample (and paternal sample) if not previously tested as above should be sent at the same time as the fetal sample

Note in people of high risk family origins for α^0 thalassaemia, there is a possibility of a hidden risk to the fetus of homozygous α^0 thalassaemia, as the carrier state for α^0 thalassaemia can be masked. It is important to determine the α genotype by DNA analysis.

Beta thalassaemias

If one biological parent carries β thalassaemia, Hb Lepore, $\delta\beta$ thalassaemia and the other carries β thalassaemia, Hb Lepore, $\delta\beta$ thalassaemia, HbO^{Arab} or HbS then:

- if time permits blood samples from both biological parents should be sent to confirm the mutations prior to fetal sampling
- if PND has been accepted, a fresh maternal sample (and paternal sample) if not previously tested as above should be sent at the same time as the fetal sample

Note in people of high risk family origins for α^0 thalassaemia, the carrier state for α^0 thalassaemia may also be present. It is important to determine the α genotype by DNA analysis.

Alpha thalassaemias

Coinheritance of α thalassaemia with other haemoglobin variants

Blood samples should not be sent to confirm and identify α thalassaemia, unless both biological parents are of high risk family origins for α^0 thalassaemia. There is rarely clinical interaction between α thalassaemia and other haemoglobin variants. Unless there are high risk family origins, the type of α thalassaemia is almost always the α^+ thalassaemia type, which poses no serious genetic risk to the fetus.

Alpha plus thalassaemia (α^+ thalassaemia or $-\alpha/\alpha\alpha$ and $-\alpha/-\alpha$)

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

Heterozygotes, ($-\alpha/\alpha\alpha$) generally have an MCH of 25 to 28pg and a normal HbA₂ level. Approximately one third of cases will be silent.

Homozygotes, ($-\alpha/-\alpha$) generally have an MCH below 25pg and some have HbH inclusions, as do carriers for α^0 thalassaemia ($--/\alpha\alpha$).

Individuals may be heterozygous for an α gene deletion (carrier genotype: $-\alpha/\alpha\alpha$) or a rarer point mutation in one gene affecting gene expression (carrier genotype: $\alpha^T\alpha/\alpha\alpha$), commonly called a non-deletional mutation. There are 2 common deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and a number of less common non-deletional mutations.

Alpha zero thalassaemia (α^0 thalassaemia (--/ $\alpha\alpha$))

This carries the potential for a clinically significant disorder. If both biological parents are carriers of α^0 thalassaemia (--/ $\alpha\alpha$), the couple has a 1 in 4 risk of having a fetus with Hb Bart's hydrops fetalis syndrome (--/--) and the mother is at 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 biological parent carries α^0 thalassaemia (--/ $\alpha\alpha$) and the other α^+ thalassaemia (either - α / $\alpha\alpha$ or - α / $-\alpha$), there is a risk of having a child with HbH disease (--/ $-\alpha$). PND is not usually indicated for HbH disease unless there are known higher risk mutations such as Hb Constant Spring.

Quality assurance and accreditation

All commissioners and service providers must refer to the public health functions agreement (Section 7A) service specification (No 18), and supporting standards and handbook to ensure a service is set up correctly and is meeting the standards set by the national screening programme.

DNA laboratories must:

- be accredited by the UK Accreditation Service (UKAS) to ISO. 'Medical laboratories – Requirements for quality and competence (ISO 15189) or be CPA accredited and actively transitioning towards ISO 15189
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UKAS will assess both the ISO and the screening requirements on behalf of PHE Screening Quality Assurance Services and the NHS Sickle Cell and Thalassaemia Screening Programme.

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Appendix 1 – Carrying out a look back with particular reference to HbA₂

A lookback is a review of screening records to identify individuals harmed or at risk of harm as a result of a screening safety incident or serious incident. Look backs or case review may also be done as part of fact finding to establish whether there has been a screening incident. Look backs may result in a patient notification exercise/recall.

If your external quality control shows the HbA₂ is out of consensus then a look back exercise must be considered. HbA₂ values which fall below consensus are of particular concern as in this situation β thalassaemia carriers may have been missed. The following steps are a guide to carrying out a look back exercise. Whilst not mathematically exact, the calculation given is suitable for this purpose. It should be noted that every situation is unique and you may wish to obtain advice from your external quality control provider and the NHS Sickle Cell and Thalassaemia Screening Programme.

If a look back exercise is required report this as a suspected safety incident in accordance with Managing safety incidents in NHS Screening Programmes. This means informing the Screening QA Service (region) and the host organisation responsible for the laboratory in addition to relevant clinical teams (including midwifery). The accountable organisation will need to decide if the Duty of Candour applies. Reference should be made to PHE guidelines on Duty of Candour (see www.gov.uk/government/publications/nhs-screening-programmes-duty-of-candour).

Method

1. Identify the point in time the results were last within consensus.
2. Look back must review patient results from that time point until results are proven to be back in consensus. If results are not yet proven to be within consensus then the actions described should continue prospectively.
3. All patient results must be reviewed, however, the following formula will help identify patients requiring more stringent scrutiny and possible recall.

Formula: Discrepancy value = consensus HbA₂ minus HbA₂ obtained by your laboratory.

Patients requiring stringent review = HbA₂ action value (3.5%) minus your calculated discrepancy value. For example: consensus = 3.6% and obtained = 3.2% therefore $3.6 - 3.2 = 0.4\%$ (discrepancy value = 0.4%). In this scenario all patients with an HbA₂ greater than or equal to 3.1% from the time point identified in (1) should be carefully reviewed.

4. Patients with an HbA₂ greater than the value identified using the above formula and with an MCH less than 27pg not already identified as a β thalassaemia carrier, should be recalled and retested.
5. Patients with an MCH greater than 27pg and with an HbA₂ which would have been greater than 4.0% when the discrepancy value identified using the above formula is added to the original result obtained should be recalled and retested.
6. All other patients do not need recalling or retesting.

Appendix 2 – Best practice guidelines for analysing liquid capillary blood samples

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 blood spot 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 blood spot screening programme, then this approach should be encouraged. Those laboratories that perform second-line testing for the newborn blood spot screening programme would already satisfy most 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. To be a ‘1 in 4’ high risk pregnancy, the haemoglobinopathy results must be known on both biological parents. 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 newborns.

1. The analytical technique for initial testing of the capillary specimen and the second line test must be appropriate for specimens from the newborn period. Some analytical protocols and instruments are designed for testing adult blood and are not optimal for newborns.
2. The laboratory must participate in an appropriate EQA scheme that assesses the analysis and interpretation of results from newborn specimens. UK NEQAS (H) provides suitable quality control specimens for this work.
3. 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.
4. Both biological 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 biological parents on the request form.
5. The laboratory analysing the liquid specimen is responsible for notifying the newborn screening laboratory of the result. There must be documented local systems in place for this process. Any discrepancies between the 2 results need




to be investigated rapidly at local level by both laboratories and reported to the NHS SCT Screening Programme and regional team within one week of the discrepancy being detected.

6. Laboratories offering this service must collate data for audit purposes on the number of specimens tested, turnaround times and consistency with newborn screening results.
7. 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.
8. 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 SOPs must include senior level checking of analytical results before reporting and follow up of the subsequent bloodspot screening result.
9. A mother and baby who are ready to be sent home should not be kept in hospital awaiting the results of these tests.
10. The laboratory must be accredited as per screening programme requirements.

Appendix 3 – Table of biological parental carrier state combinations

Biological father	Carrier of:	HbS	β thalassaemia	Hb Lepore	HbE	HbO ^{Arab}	HbC	HbD ^{Punjab}	HPFH	Not identified as a carrier
	HbS	Yellow	Yellow	Pink	Pink	Pink	Yellow	Yellow		
	β thalassaemia	Yellow	Yellow	Yellow	Yellow	Yellow				
	$\delta\beta$ thalassaemia	Pink	Yellow	Pink						
	Hb Lepore	Pink	Yellow	Yellow						
	HbE	Pink	Yellow	Pink						
	HbO ^{Arab}	Yellow	Pink	Pink						
	HbC	Yellow								
	HbD ^{Punjab}	Yellow								
	HPFH	Pink								
	Not identified as a carrier									

Key

	Serious risk - refer couple for counselling - prenatal diagnosis to be offered
	Less serious risk - refer couple for counselling - further investigation may be required
	Minimal risk

Useful organisations and websites

The Oxford Laboratory provides, on behalf of the screening programme, a dedicated sickle cell and thalassaemia laboratory support service for antenatal and newborn laboratories and other healthcare professionals involved in the screening programme.

Telephone: 01865 572 767

Email: lab.support@nhs.net

FAX: 01865 572775

NHS Sickle Cell and Thalassaemia Screening Programme enquiries:

Telephone: 020 3682 0890

Email: Phe.screeninghelpdesk@nhs.net

Website: gov.uk/phe/screening

[British Committee for Standards in Haematology](#)

[SCT screening e-learning for health care professionals](#)

[Laboratory e-learning modules](#)

[NHS Choices: screening for sickle cell and thalassaemia](#)

[National Sickle Cell/Thalassaemia Centres](#)

[Family origin questionnaire](#)

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About PHE Screening

Screening identifies apparently healthy people who may be at increased risk of a disease or condition, enabling earlier treatment or better informed decisions. National population screening programmes are implemented in the NHS on the advice of the UK National Screening Committee (UK NSC), which makes independent, evidence-based recommendations to ministers in the four UK countries. The Screening Quality Assurance Service ensures programmes are safe and effective by checking that national standards are met. PHE leads the NHS Screening Programmes and hosts the UK NSC secretariat.

PHE Screening, Floor 2, Zone B, Skipton House, 80 London Road, London SE1 6LH
www.gov.uk/topic/population-screening-programmes
Twitter: [@PHE_Screening](https://twitter.com/PHE_Screening) Blog: phescreening.blog.gov.uk

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