Most haematology laboratories that undertake antenatal haemoglobinopathy screening also undertake pre-anaesthetic sickle screening, and sometimes newborn screening, in the same section, by the same people using the same equipment, and the results may end up in the same patient's notes. It is important, therefore, that the report makes clear which conditions have been tested for. Although many synonyms are available, it is important that all laboratories report similar data in a similar way, in order to avoid causing confusion to clinicians and patients. The recommendations below refer to the common screening procedures and do not include specialised tests such as mass spectrometry or DNA analysis.

With over 800 mutations responsible for the production of variant haemoglobins and over 300 mutations responsible for the thalassaemia syndromes, it is usually not possible to identify the precise protein structure or mutation without detailed protein or DNA analysis. However, it is usually possible to detect the presence of the common medically significant haemoglobin (Hb) variants and most of those people who are β-thalassaemia carriers by simple laboratory techniques and to identify them with sufficient accuracy for clinical purposes. On the rare occasions when the precise amino acid structure or DNA mutation is required then detailed protein or DNA analysis must be undertaken.

The presence of the uncommon but clinically important variants such as the unstable or high-affinity haemoglobins can be detected best by the functional tests of haemoglobin stability and oxygen affinity, respectively, and the Hb M's by electrophoresis at pH 7 or by spectroscopy. In all cases, however, detailed protein or DNA analysis is still needed to identify the precise Hb variant involved. The diagnosis of α-thalassaemia will usually need to be made by exclusion of iron deficiency and β-thalassaemia in a person with hypochromic, microcytic red cell indices. In some circumstances, however, DNA analysis will be essential.

These recommendations (including examples on the website) on reporting analytical data and the genetic implications (conclusions) on that data should provide sufficient information to allow all normal and over 95% of abnormal results to be reported in a standardised manner. However, because of the diversity of Hb variants and thalassaemia syndromes, there will always be some situations that require further tests on different samples or require family studies before a useful clinical diagnosis can be obtained. Individual laboratories may have to make minor changes to the ‘normal, borderline and raised’ cut-off limits for Hb A2 quantitation. Hopefully, when the wider use of national and international standards is adopted, all laboratories will have the same reference ranges for Hb A2 as they already do for blood counts.

It is important to realise that almost all haemoglobinopathy results are used to 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. In particular, they may affect the choice of partner(s) and whether or not a couple decide to have children, and, if so, whether to consider prenatal diagnosis. In this situation it is good practice for two people to assess the data and conclusions of all abnormal results before the reports are authorised, as this will reduce the incidence of misdiagnosis.
If an individual has had a blood transfusion and any of the transfused red cells remain in the circulation then misleading data and conclusions may result. Thus, it is essential that clinicians realise this and it may be prudent to have a universal footnote on all haemoglobinopathy results such as: This data and the interpretation of the data may be misleading if the patient has had a recent transfusion. If so, the tests should be repeated at least four months after the last transfusion. The small proportion of results 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, liver disease or B_{12}/folate deficiency. Such results will need to be discussed with a senior clinician or clinical scientist to decide what further tests or family studies are indicated to elucidate the laboratory data and the request posed by the clinician caring for the patient. In many situations, family studies and knowledge of the patient’s haematological status will prove extremely helpful.

Notes on reporting

1. The condition tested for must be made clear to the clinician (eg antenatal sickle cell and thalassaemia screening, pre-anesthetic sickle screening or newborn screening). For instance, if only sickle screening has been undertaken then the result is quite appropriate for an anaesthetist to make a decision about the clinical peri-operative care but it is inadequate for genetic counselling.

2. Give sample date (this can be extremely important if a person has had a recent blood transfusion).

3. Separate analytical fact from interpretative opinion. First, give the factual results and then give a clear conclusion, which may include recommendations. If there is likely to be a delay in producing a final result, consider giving an interim result, which may be sufficient for the clinician to move forward with the patient’s clinical care.

4. The blood count should always be reviewed as it may be the only indication of α-thalassaemia and a raised red cell distribution width (RDW) may flag up a complicating situation. The blood film should be available, and is especially important if the RDW is abnormal. It can sometimes provide essential information, for instance to detect a transfused homozygous sickle cell anaemia (Hb SS) person who can otherwise appear like a sickle cell carrier (Hb A + Hb S) in other analytical procedures. If the blood film is inconsistent with the other analytical data it may indicate a specimen mix up. Examination of the blood film can therefore reduce errors.

5. If information from the blood count is made up of 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 (eg RBC, M CH, M CV) should be included in the haemoglobinopathy report. This is to enable the clinician looking after the individual to review the blood count for consistency with previous data that may be available to them but not to the haemoglobinopathy laboratory.

6. Similarly, if information on ethnicity is made up of it should be stated in the report.

7. If the sickle solubility test is positive it should only be reported as an ‘interim’ report. The final report with information from the blood film, high-performance liquid chromatography (HPLC) and/or electrophoresis and any other appropriate tests should follow as soon as possible.

8. The sickle solubility test may be negative in newborns who are carriers for sickle cell, or even those with sickle cell disease. This is due partly to the smaller amount of Hb S present and partly to the larger amount of Hb F in newborns. For this reason, a negative sickle solubility test should be viewed with caution in children less than six months of age. Haemoglobin electrophoresis (at alkaline and acid pH) or HPLC or isoelectric focusing (IEF), or a combination of these, should be used to assess the sickle status in children under six months of age.

9. There are many D-like and G-like haemoglobins (about 30) but only Hb D^\text{+}\text{+}\text{+}\text{+}\text{+} is clinically significant. If a specific test (DNA or mass spectrometry) for Hb D^\text{+}\text{+}\text{+}\text{+}\text{+} is not readily available then consideration should be given to undertaking alkaline globin electrophoresis to differentiate an α-globin variant from a β-globin variant, as this will help elucidate the clinical significance of the variant.

10. Isoelectric focusing can be used as an alternative to cellulose acetate electrophoresis (CAM) at alkaline pH, but not as an alternative to acid agarose or citrate agar; however, it can of course be used to complement these tests. Cellulose acetate electrophoresis relies on charge change for separation and in general gives similar mobility to IEF, although IEF does show better resolution than CAM. Citrate agar (and acid agarose) relies not only on charge but also on solubility of the different haemoglobins in agar.

11. The elution time of the haemoglobin peaks in HPLC analysis is very dependent on temperature, and changes may lead to mislabelling of peaks. It is therefore extremely important that chromatograms are inspected carefully before authorising the results. This is especially important if the equipment is interfaced to laboratory computers that produce a report. The elution times are likely to be different with different columns and buffers, and between different manufacturers.

12. Automated HPLC will usually give reliable results for Hb A\text{2} and Hb F quantitation but may give misleading results in the presence of a haemoglobin variant. If quantitation of Hb A\text{2} is needed in this situation it should be measured by electrophoresis and elution or using microcolumns that are specific to this task. This is a particular problem with sickle haemoglobin as post-translational adjuncts often elute in the same places as Hb A\text{2} and Hb A. In homozygous Hb SS disease this can lead not only to the appearance of a falsely raised Hb A\text{2} but also to the apparent presence of Hb A, resulting in the patient being misdiagnosed as Hb A\text{2} θ-thalassaemia. Similarly, some variants elute in the same position as Hb F and if an accurate quantitation of Hb F is needed in this situation a different technique (such as the two-minute alkali denaturation technique) should be used.

13. The Hb F can be raised as a normal response to pregnancy. If the Hb F is 1–4% in a pregnant woman this should be noted as ‘raised Hb F, but compatible with pregnancy’, unless the laboratory computer can be programmed to give an appropriate reference range for samples from pregnant women.

14. Severe iron deficiency anaemia with a haemoglobin level less than 8 g/dL may sometimes lower the Hb A\text{2} (by up to 0.5%). Hence, if the Hb A\text{2} is borderline, or 3.0–3.5% consider correcting the iron deficiency and repeating the Hb A\text{2} before making a final decision about whether or not an individual is a carrier for β-thalassaemia. There may not be time to do this during pregnancy and it may be more appropriate to assume the woman is at risk of being a carrier for β-thalassaemia and thus test the partner.

15. θ-thalassaemia and the related Hb Barts Hydrops are very rare outside certain ethnic communities, which include those in South-East Asia (including southern China) and the eastern Mediterranean (Greece, Cyprus and Turkey). In screening situations, these are the ethnic groups that should be considered at risk for θ-thalassaemia.

16. Hypochromic, microcytic red cell indices are compatible with carriers for α-or β-thalassaemia or iron deficiency, or any combination of these three. As a general rule, the red count is relatively higher in relation to the haemoglobin and M CH in carriers of thalassaemia than that found in iron deficiency, but this may not happen in pregnancy or iron-deficient PRV. It is clinically helpful to family doctors and general physicians to flag up these possibilities if the M CH is reduced (say <27 pg in adults). The M CV can rise considerably with storage of a blood sample and so should only be used with caution in the assessment of thalassaemia unless the length of time and storage...
temperature between venesection and analysis is known. Although a raised Hb A₂ is a marker for carriers of β-thalassaemia (see point 12 above) it is not usually possible to be specific about the presence of iron deficiency or α-thalassaemia. Unless information is available about the individual’s clinical state at the time the blood sample was taken, the apparent iron status may be misleading because many medical situations can interfere with measurements of iron status. When considering the genetic implications in situations such as pregnancy, an MCH of < 25 pg can be used as an action flag for α-thalassaemia because this condition is thought to be very rare if the MCH is > 25 pg. Iron deficiency or α-thalassaemia can occur at MCH levels above this, but they do not have the same genetic or obstetric implications as α-thalassaemia. In practice, it is reasonable to comment on the possibility of iron deficiency and/or α-thalassaemia trait (carrier) if the MCH is < 27 pg and the Hb A₂ is not raised (but see 14 above). α-thalassaemia should only be considered in screening if the MCH is < 25 pg and the individual is of SE Asian, Greek, Turkish or Cypriot ethnic origin (see 15 above).

17 In relation to antenatal and preconceptional screening, partner testing should be recommended whenever a person has been identified as a sickle carrier or a carrier for some other condition that interacts with sickle haemoglobin or thalassaemia to cause clinical problems. Partner testing should also be recommended if a carrier has been detected but the clinical significance is unknown. The decision about whether or not to offer partner testing to an individual should rest with the clinician who will know the clinical situation, including whether or not the partner has already been tested and whether or not there has been a miscarriage.

18 The conclusion should always be given both in full text and in standard abbreviated form in parentheses, as this improves clarity. For example: sickle cell carrier (Hb A + Hb S) or homozygous sickle cell anaemia (Hb SS).

19 Consider using the following footer on all reports: Any genetic predictions following analytical facts and the conclusion should only be considered if the MCH is > 25 pg. Iron deficiency or α-thalassaemia because this condition is thought to be very rare if the MCH is > 25 pg. Iron deficiency or α-thalassaemia can occur at MCH levels above this, but they do not have the same genetic or obstetric implications as α-thalassaemia. In practice, it is reasonable to comment on the possibility of iron deficiency and/or α-thalassaemia trait (carrier) if the MCH is < 27 pg and the Hb A₂ is not raised (but see 14 above). α-thalassaemia should only be considered in screening if the MCH is < 25 pg and the individual is of SE Asian, Greek, Turkish or Cypriot ethnic origin (see 15 above).

20 With more than 800 haemoglobin variants that can interact with each other and also with α- and/or β-thalassaemia, it is not usually possible to arrive at an absolutely precise diagnosis unless mass spectrometry of the haemoglobin variant or DNA analysis is undertaken. However, if an Hb variant is detected and the laboratory analyses include techniques that rely on at least three different biological or chemical properties, it is usually possible to make a diagnosis that is precise enough for clinical purposes. For example, a sample with a positive sickle test, two peaks (or bands) eluting (or migrating) with Hb A and Hb S (with the Hb S less than 45% of the total haemoglobin), and a blood film showing normal red cell morphology, it is reasonable to conclude that the individual is a sickle cell carrier (Hb A + Hb S). If it is not possible to use at least three different techniques, each relying on a different property – as occurs with newborn screening from Guthrie cards – then the conclusion should be prefaced by ‘probable’ (eg probable sickle cell carrier [Hb F + Hb A + Hb S]). In those instances where a precise genetic diagnosis is needed then mass spectrometry or DNA analyses will need to be undertaken. If a liquid blood sample can be obtained, these specialised tests may not be necessary.

21 An Hb A₂ of 3.5–3.7% by HPLC should be considered a borderline value and many people with such a result will not be carriers for β-thalassaemia. However, if a member of the couple is pregnant then the partner should be tested as soon as possible. If the partner is found to be a carrier for β-thalassaemia or sickle haemoglobin (or an interacting haemoglobin, see below) then a sample from both partners should be referred for DNA analysis.

22 If a member of a couple is found to have β-thalassaemia or sickle haemoglobin, or a haemoglobin variant that is known to interact with β-thalassaemia or sickle haemoglobin, then the partner should be tested. If a member of the couple is pregnant then such testing should be undertaken as soon as possible. The haemoglobin variants to be considered in this situation are Hb C, D₇°⁺, E, O, Lepore and any unusual haemoglobin with a positive sickle test. Some laboratories may have difficulty distinguishing Hb D₇°⁺ from the many D-like and G-like variants. If a member of the couple is pregnant, or contemplating pregnancy, then such samples should be referred to a specialist laboratory that is able to determine the precise nature of the haemoglobin variant by other techniques such as DNA or mass spectrometry.

23 In conclusion, ensure that both the analytical facts and the conclusion message are clear so that the report will lead to the action you consider necessary and not result in inappropriate worry.

**Example reports**

In the example reports that follow, words in parentheses include alternatives. For example: ‘HPLC (or CAM)’ with a peak (band) indicates a haemoglobin peak with HPLC or a band with CAM. Text in italics represents directions for the laboratory and are not intended to be included in the report. Note: the national guidelines now recommend the use of ‘carrier’ instead of ‘trait’.

**Pre-operative sickle screening**

**EXAM PLE 1**
Sickle solubility test is negative and/or HPLC (or CAM) only shows a single major Hb band (peak) with Hb A.

**Analytical data**
Sickle solubility test: Negative.
HPLC (or Hb electrophoresis at alkaline pH): One major peak (band) with Hb A.

**Conclusion**
No evidence of sickle haemoglobin.

**EXAM PLE 2**
Sickle solubility test is positive, CAM (or HPLC) shows two major bands (peaks) with Hb A and Hb S, with Hb A > Hb S (Hb S > 30% of the total haemoglobin), and the blood film shows normal red cell morphology.

**Analytical data**
Sickle solubility test: Positive.
HPLC (or CAM): Two major peaks (bands) with Hb A and Hb S.
Hb S: 34% (if quantitated).

**Conclusion**
Sickle cell carrier (Hb A + Hb S).

**EXAM PLE 3**
Sickle solubility test is negative and/or CAM (or HPLC) only shows a single major Hb band (peak) with Hb A, and the Hb A₂ is raised (> 3.7%) and FBC shows an MCH < 27 pg.

**Analytical data**
Sickle solubility test: Negative.
HPLC (or CAM): One major peak (band) with Hb A.
Hb A₂: 5.2% (normal range [NR] 2.0–3.3%).

**Conclusions**
1) β-thalassaemia carrier.
2) No evidence of sickle haemoglobin.

**EXAM PLE 4**
Sickle solubility test is positive, CAM (or HPLC) shows a single major band (peak) with Hb S, acid agarose (citrate agar)
shows a single major band with Hb S, and the blood film shows red cells consistent with homozygous sickle cell anaemia.

**Analytical data**
Sickle solubility test: Positive.
HPLC (or CAM): A major peak (band) with Hb S.
Hb electrophoresis at acid pH (citrate agar or acid agarose): one band with Hb S.
Conclusion
Homzygous sickle cell anaemia (Hb SS).

Antenatal and preconceptional screening for sickle cell and thalassaemia

EXAM PLE 1
HPLC (or CAM) only shows a single major Hb peak (band) with Hb A, and the Hb A2 is normal (< 3.5%), and the Hb F is normal (< 1%), and red cell indices are normal and the Hb is > 8 g/dL.

Analytical data
HPLC (or CAM) at alkaline pH: One major peak (band) with Hb A.

Hb A2: 2.5%

Hb F: 0.7%

Conclusions
1) No evidence of sickle haemoglobin or other common haemoglobin variant and no evidence of thalassaemia carrier.
2) No action needed.

If the Hb F is slightly raised (1-4%), add ‘Hb F raised but consistent with pregnancy’ to report.

EXAM PLE 2
HPLC (or CAM) shows two major peaks (bands) with Hb A and Hb S, sickle solubility test is positive, Hb A > Hb S (Hb S > 30% of the total haemoglobin), the RDW is normal and the blood film shows normal red cell morphology.

Analytical data
Sickle solubility test: Positive.

HPLC (or CAM): Two peaks (bands) with Hb A and Hb S.

Hb S: 34%

Conclusions
1) Sickle cell carrier (Hb A + Hb S).
2) No evidence of thalassaemia
3) Partner testing recommended.

EXAM PLE 3
HPLC (or CAM) shows one major peak (band) with Hb S and acid agarose (citrate agar) shows one major band with Hb S, sickle solubility test is positive, and the blood film shows red cells consistent with homozygous sickle cell anaemia.

Analytical data
Sickle solubility test: Positive.

HPLC (or CAM): One peak (band) with Hb S.

Analysing cell morphology shows red cells with grey cytoplasm and the blood film shows normal red cell morphology.

Conclusions
1) Homzygous sickle cell anaemia (Hb SS).
2) Partner testing recommended.

EXAM PLE 4
HPLC (or CAM) only shows a single major Hb band with Hb A and A2 is raised (> 3.7%) and FBC shows an MCH < 27 pg.

Analytical data
HPLC (or CAM): One major peak (band) with Hb A.

Hb A2: 5.2% (NR 0.2%-3.3%)

Conclusions
1) Beta-thalassaemia carrier.
2) No evidence of sickle haemoglobin.
3) Partner testing recommended.

EXAM PLE 5
HPLC (or CAM) only shows a single major Hb peak (band) with Hb A, the Hb A2 is normal (< 3.5%), Hb is > 8 g/dL and FBC shows an MCH < 25 pg.

Analytical data
Sickle solubility test: Negative.

HPLC (or CAM): One major peak (band) with Hb A.

Hb A2: 2.5%

Conclusions
1) No evidence of sickle haemoglobin or other common haemoglobin variant, or alpha-thalassaemia carrier.
2) In view of RBC indices (Hb g/dL, RBC x10/L, MCV fl, MCH pg), possible iron deficiency and/or alpha-thalassaemia carrier.
3) Partner testing recommended if either member of the couple is of SE Asian or Eastern Mediterranean (Cypriot, Greek or Turkish) origin.

Newborn screening
Hospital newborn screening for sickle cell disease and beta-thalassaemia major is usually performed using umbilical cord blood.

Newborn screening, in conjunction with that for phenylketonuria, congenital hypothyroidism and certain other inherited diseases, using dried blood spots will be reported differently in England in conjunction with child health services.

EXAM PLE 1
HPLC (or CAM or IEF) shows two major Hb peaks (bands) with Hb F and Hb A.

Analytical data
HPLC (or CAM or acid agarose or IEF): Two major peaks (bands) with Hb F and Hb A.

Conclusions
1) Probable sickle cell carrier.
2) Suggest referral to a paediatric sickle cell clinic.

Note: Hb F is normal in newborns.

Example reports online
A comprehensive list of example reports for use in pre-operative, antenatal and preconceptional, and newborn screening situations can be found on the IBMS website (see below).

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FURTHER INFORMATION
- www.ibms.org/
- www.kcl-phs.org.uk/haemscreening

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