Guidance notes for Haematology laboratories in England for the referral of antenatal patient samples to the DNA laboratories for haemoglobinopathy mutation analysis.

These guidance notes have been produced by NHS Sickle Cell & Thalassaemia Screening Programme Laboratory Subgroup to help antenatal screening laboratories identify which samples need further analysis by mutation identification for the purpose of assessing or confirming the risk of a couple having a baby affected by a severe haemoglobin disorder.
The majority of couples at risk of having a child affected with beta thalassaemia or sickle cell disease are identified initially by laboratory haematological techniques through the antenatal screening programme. The diagnosis of alpha thalassaemia is more complicated because of the need to exclude iron deficiency and because DNA analysis is the only precise way to distinguish between the two classes of alpha thalassaemia – alpha plus (α^+) and alpha zero (α^O) thalassaemia. However it is not physically possible to confirm all potential cases of alpha thalassaemia by DNA analysis because the alpha plus form is too common and the DNA labs do not have the resources. Furthermore, non-deletion forms of alpha plus thalassaemia are more common than was thought and rapid methods for their detection are not available. A strategy for diagnosis of alpha thalassaemia by combining haematological and details of ethnic origin is proposed in this document. This should limit the number of samples requiring referral for DNA analysis of alpha thalassaemia.

These DNA referral guidelines are based on the antenatal screening guidelines proposed by the NHS Sickle Cell & Thalassaemia Screening Programme Committee for the national antenatal screening programme. *It must be emphasised that the antenatal screening programme guidelines are designed only to identify most carriers for sickle cell disease, thalassaemia and related disorders; the screening protocol will not lead to the identification of every couple at risk for every haemoglobinopathy.* For example they are not designed to pick up couples at risk for Hb H disease, or the extremely rare cases of Asian Indian couples at risk for Hb Bart's Hydrops fetalis syndrome. Antenatal screening laboratories may upgrade the guidelines for partner screening to take into account of specific rare haemoglobin disorders that might occur in their local population after consultation with regional centres.

These guidance notes should be used in conjunction with:

a) The guidance notes for antenatal screening issued by the NHS Haemoglobinopathy Screening Programme Laboratory Subgroup: "Reporting Haemoglobinopathy Results – Antenatal Screening"

b) published guidelines for haemoglobinopathy screening (Appendix 2)

1. Genetic disorders

Antenatal screening is required to detect the carrier state (trait) for several common haemoglobin variants, beta thalassaemia and alpha thalassaemia. In particular these are Hb S, C, E, D Punjab, O Arab, Lepore, α^+, α^o, β, δβ -thalassaemia and HPFH. These carrier states, except HPFH, when co-inherited may interact to produce mild or severe clinical disorders as described in the table listed in Appendix 1.

For example: if the mother is a carrier for Hb S, there is a genetic risk of a couple having a child affected with a serious disorder (sickle cell disease) if the partner is a carrier of Hb S, β-thalassaemia, Hb O-Arab, Hb C, Hb D-Punjab. There is a risk of a less seriously affected pregnancy if the partner is a carrier for δβ-thalassaemia or Hb Lepore, and no risk if the partner is a carrier for HPFH, Hb E or α-thalassaemia.

Note: Carriers of the various β-thalassaemias who are also of Southeast Asian or Mediterranean ancestry may also carry α^{O}-thalassaemia, and thus have a hidden risk if their partner also carries α^{O}-thalassaemia.

Patient Information: Information materials on haemoglobin disorders for counsellors can be downloaded from the web site http://www.chime.ucl.ac.uk/APoGI/. The site contains information for health professionals, information for carriers and information for couples where one or both partners are carriers, including genetic risks of globin gene disorder combinations and information about prenatal diagnosis for serious inherited disorders.

2. Antenatal Screening of Maternal Blood Sample

The NHS Haemoglobinopathy Screening Programme Laboratory Subgroup guidelines for the screening of maternal blood (Appendix 2) list 10 outcomes for the reporting of a maternal carrier phenotype, as summarised in the table below.

Conclusion for maternal carrier state	**Action required**	**Possible risk to pregnancy**
Normal	Partner testing not required	none
Hb S	Suggest test partner	Sickle cell disease
Hb C	Suggest test partner	Sickle cell disease
Hb D-Punjab	Suggest test partner	Sickle cell disease
Hb E	Suggest test partner	Thal major / intermedia
β-thalassaemia (Hb A_2 of 3.5% and above)	Suggest test partner	Thal major/intermedia / sickle cell disease.
α-thalassaemia	Suggest test partner if MCH is below 25pg and both partners of SE Asian or Med origin*.	Hb Bart's Hydrops fetalis Syndrome
Hb A_2 variant (split Hb A_2 peak on HPLC)	Partner testing not required if total Hb A_2 is less than 3.5%**	Thalassaemia major / intermedia
Other results***	Refer results for a Consultant expert opinion	Thalassaemia, sickle cell disease, or severe haemolytic anaemia

* Note: the antenatal screening programme policy will not detect couples at risk of having a child with Hb H disease

** Note: the antenatal screening programme policy will not detect some patients with normal Hb A_2 β-thalassaemia. These mutations are associated with reduced red cell indices and a Hb A_2 value in the range of 3.3-3.8%.).

*** Other maternal phenotypes that indicate partner testing are the carrier states for Hb O-Arab, δβ-thalassaemia and Hb Lepore trait (see genetic risk table). Rare unstable haemoglobin variants, high

affinity variants or Hb Ms may interact with thalassaemia that affects the same chain as the variant thus increasing its concentration greatly, to produce a severe disorder. Partner testing is thus indicated with these rare phenotypes also.

3. Partner testing

This is done by the same haematological testing strategy as for maternal phenotype testing. If the partner has a haemoglobinopathy that can interact with the maternal phenotype as depicted in the table, then the couple should be counselled and, if the parents so choose, fresh blood samples sent to a DNA referral laboratory with appropriate consent for molecular analysis in preparation for prenatal diagnosis according to the following guidelines.

If the partner is unavailable for testing, a risk assessment should be done. If indicated the woman should be offered prenatal diagnosis. Prenatal diagnosis for sickle cell disease can be undertaken without the partner's DNA. Similarly, prenatal diagnosis for β-thalassaemia can be undertaken without the partner's DNA, although the diagnosis will not be able to be given with a 100% certainty if the partner's mutation is not known.

4. Guidelines on the referral of blood samples for DNA Analysis

Hb Variants

If one partner carries:

Hb S

a) *and the other carries Hb S*:
Blood samples are not required immediately on carrier identification to confirm Hb S trait by DNA analysis. Once prenatal diagnosis (PND) has been chosen, fresh maternal and paternal blood samples should be referred at the same time as the fetal tissue sample is referred.

b) *and the other carries Hb C*:
Blood samples should not be sent to confirm Hb C by DNA analysis. The same guidelines apply as for HbS above.

c) *and the other is thought to carry Hb O-Arab, Hb D-Punjab, Hb Lepore or a type of β-thlassaemia trait*:
Both maternal and paternal blood samples should be referred with appropriate consent for mutation analysis, even if PND is not required. In the latter case, DNA analysis will confirm the genetic risk and predict its severity for counselling purposes, and the DNA will be stored to aid future genotype analysis of children.

d) *and the other carries deletional HPFH*:
Both maternal and paternal blood samples should be referred with appropriate consent for mutation analysis, even if PND is not required. This is to identify the HPFH mutation and to confirm the absence of genetic risk for counselling purposes.

Note: Carriers of deletional HPFH have a raised Hb F level of 20-30%. There are two types found only in individuals of African origin, the HPFH1 deletion and the HPFH2 deletion, called the Ghanaian deletion. It is important to differentiate deletional HPFH from δβ-thalassaemia. The carrier state for δβ-thalassaemia is associated with reduced red cell indices and a Hb F level usually in the

range of 5-15%. Nondeletional HPFH (the heterocellular form) usually results in a more modest increase of Hb F (1-5%) in adults and is found in many populations. This type of HPFH is not normally tested for by DNA analysis.

Hb S with α-thalassaemia

Blood samples should not be sent to confirm and identify α-thalassaemia, unless there is a real risk that is the α^{o}-thalassaemia type (only in patients with Hb S of East Mediterranean origin, ie Cyprus, Greece or Turkey). The same guidelines for referral apply as for Hb S above, for any combination of partner carrier status. There is no interaction of Hb S with α-thalassaemia and the genetic risks are the same as for Hb S without α-thalassaemia. The type of α-thalassaemia found in Hb S patients of Afro-Caribbean origin is almost always the α^{+}-thalassaemia type, which poses no serious genetic risk to the fetus.

Hb C

The other carries Hb S:
DNA analysis is not necessary to confirm Hb S or Hb C. Fresh maternal and paternal blood samples need to be sent only when prenatal diagnosis (PND) is required. The parental samples can be sent at the same time as the fetal tissue sample.

Hb D

The other carries Hb S:
Fresh maternal and paternal blood samples should be referred with appropriate consent for Hb D identification by mutation analysis even if PND is not required. This is to confirm the genetic risk of sickle cell disease from Hb S/Hb D-Punjab, as other types of Hb D do **not** interact with Hb S to cause sickle cell disease.

Hb E

***a) The other carries β-thalassaemia*:**
Both maternal and paternal blood samples should be referred with appropriate consent for mutation analysis, even if PND is not required. This is to confirm the genetic risk and predict its severity for counselling purposes and to aid future genotype analysis of children. There is also the possibility of a hidden risk for α^{o}-thalassaemia, as this can be masked in individuals of South East Asian origin carrying Hb E or β-thalassaemia.

b) The other carries δβ-thalassaemia* or *Hb Lepore:
Both maternal and paternal blood samples should be referred with appropriate consent for mutation analysis, even if PND is not required. This is to confirm the genetic risk and predict its severity for counselling purposes and to aid future genotype analysis of children

c) The other carries α^{o}-thalassaemia:
There is a possibility of a hidden risk for homozygous α^{o}-thalassaemia, as the carrier state for α^{o}-thalassaemia can be masked in individuals carrying Hb E in individuals of South East Asian origin.

Hb O Arab

a) The other carries Hb S:
Fresh maternal and paternal blood samples may be referred for Hb O-Arab identification by mutation analysis even if PND is not required. This is to confirm the genetic risk of sickle cell disease from Hb S/Hb O-Arab.

***b) The other carries β-thalassaemia*:**
Fresh maternal and paternal blood samples should be referred with appropriate consent for mutation analysis even if PND is not required. This is to positively identify Hb O-Arab and to confirm the genetic risk of genotype Hb O-Arab/β-thalassaemia for counselling purposes,

Hb Lepore

The other carries β-thalassaemia, Hb Lepore, Hb S, Hb E, or Hb O-Arab:
Fresh maternal and paternal blood samples may be referred for mutation analysis even if PND is not required. This is to positively identify Hb Lepore and confirm the genetic risk for counselling purposes.

β-thalassaemias

a) when both partners are β-thalassaemia carriers:
Fresh maternal and paternal blood samples should be referred with appropriate consent for mutation analysis even if PND is not required. This is to identify the β-thalassaemia mutations and to determine the severity of genetic risk for counselling purposes. Ideally, identification of the β-thalassaemia mutations should be carried out before fetal tissue sampling.

Note: In people of Mediterranean (Cyprus, Greece or Turkey) or South East Asian origin the carrier state for α^{o}-thalassaemia trait may also be present, as this can be masked by the carrier state for β-thalassaemia phenotype. It is important to determine the alpha genotype by DNA analysis in such couples.

- **Normal Hb A_2 β-thalassaemia**: These mutations are associated with reduced red cell indices and a raised Hb A_2 that can be sometimes just be borderline-raised in the range of 3.3-3.8%. In combination with a β^{0}-thalassaemia mutation, these mutations result in a disorder ranging in severity from thalassaemia intermedia to thalassaemia major. A cut off value of 3.5% for the Hb A_2 level will miss some cases of normal Hb A_2 β-thalassaemia. In the UK, this is mostly due to the mutation CAP+1 A-C in Asian-Indians and IVSI-6 T-C in Mediterraneans.
- **Silent β-thalassaemia**: carriers for this will not be picked up by any screening policy. The carrier state for this type of β-thalassaemia is associated with normal red cell indices plus a normal Hb A_2 below 3.3%, (eg. as caused by the rare Mediterranean β^{+}-thalassaemia mutation –101 C-T). However this type of β-thalassaemia has such a mild phenotype that prenatal diagnosis is never indicated.

b) when one partner carries β-thalassaemia and the other carries Hb Lepore, Hb S, Hb E, or Hb O-Arab:
As detailed above

c) when the other partner carries probable or definite α^{o}-thalassaemia
Fresh maternal and paternal blood samples should be referred for *α^{o}-thalassaemia* mutation analysis if the couple are of East Mediterranean (Cyprus, Greece or Turkey) or Southeast Asian origin. The

couple may be at risk for homozygous α^{o}-thalassaemia as the carrier state for β-thalassaemia can mask the co-inheritance of α^{o}-thalassaemia.

δβ-thalassaemia

When one partner is suspected of having carrying δβ-thalassaemia and the other carries β-thalassaemia, Hb Lepore, Hb S, Hb E, or Hb O-Arab:
Both maternal and paternal blood samples should be referred with appropriate consent for mutation analysis, even if PND is not required. This is to confirm the genetic risk and predict its severity for counselling purposes.

α-thalassaemia

Brief background notes:

Alpha plus thalassaemia (α^{+}-thalassaemia)

It is found in all ethnic groups, with a high (10-30%) carrier frequency in some parts of Africa and South Asia. If both partners are carriers, there is no risk to the fetus. Homozygous α^{+}-thalassaemia is not a clinically significant disorder with respect to genetic or obstetric complications, but can cause diagnostic confusion with α^{0}-thalassaemia trait or iron deficiency.

- Heterozygotes (carriers) generally have a MCH of 25-28 pg and a normal Hb A_2 level. Approximately one third of cases will be silent.
- Homozygotes generally have a MCH below 25pg, the same as carriers for α^{0}-thalassaemia.

Mutations can be a deletion of one alpha gene (carrier genotype: -α/ αα) or a point mutation in one gene affecting gene expression (carrier genotype: $\alpha^{T}\alpha/\alpha\alpha$), commonly called a non-deletion mutation. There are two common deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and a number of less common non deletion mutations. Usually, only the deletions are tested for routinely (although at UCLH some non deletion mutations are tested for)

Alpha zero thalassaemia (α^{0}-thalassaemia)

This carries the potential of a clinically significant disorder. If both parents are carriers of alpha zero thalassaemia, the couple is at risk of having a fetus with Hb Bart's hydrops fetalis syndrome and the mother runs the risk of obstetric complications, particularly in the third trimester of pregnancy. The mutations are almost always due to a gene deletion (carrier genotype: --/αα).

If one partner carries α^{0}-thalassaemia and the other α^{+}-thalassaemia, then the couple is at risk of having a child with HbH disease. Prenatal diagnosis is not usually indicated for HbH disease.

- All heterozygotes generally have a MCH below 25pg. Unpublished studies have shown 99% of cases have a MCH < 25pg. Many have rare red cells containing Hb H inclusions but they are not always detectable by routine screening.

- α^{0}-thalassaemia is found in patients of East Mediterranean (Cyprus, Greece or Turkey) and Southeast Asian origin. There are two Mediterranean and three Southeast Asian deletion mutations, all of which can be diagnosed quickly by PCR analysis.

- It is rarely encountered in patients of African, Pakistani and Indian origin.

- Only three Asian couples at risk of Hb Bart's hydrops fetalis are known, and no African case has been reported.

- It is occasionally observed in patients of British origin. However no couple at risk of Hb Bart's hydrops fetalis has been reported. The most commonly seen mutation is the British α^0-thalassaemia mutation.

Screening policy

When the MCH is <27pg but >25pg.

If the woman has an MCH of 25-27pg, and a normal Hb A_2 (below the recommended normal cut off point, 3.5%) and a normal Hb F (<3% if pregnant), it is unlikely to be of clinical significance. This could be iron deficiency. If the patient's iron status is normal, a putative diagnosis of heterozygous α^+-thalassaemia can be made without confirmation by DNA testing**.**
The antenatal screening guidelines recommend that the partner need not be tested for any ethnic group.

a) African, Pakistani, or Indian origin

A diagnosis of heterozygous α^+-thalassaemia trait indicates the couple is most unlikely to be at risk of Hb Bart's hydrops fetalis syndrome.
The NSC guidelines indicate that the partner need not be tested.

b) East Mediterranean or Southeast Asian origin.

A diagnosis of probable heterozygous α^+-thalassaemia indicates the couple is most unlikely to be at risk of Hb Bart's hydrops fetalis syndrome.
The NSC guidelines indicate that the partner need not be tested.

When the MCH is <25pg:

If the woman has an MCH below 25pg and the Hb A_2 and Hb F values are normal, the diagnosis could be iron deficiency, homozygous α^+-thalassaemia, heterozygous α^0-thalassaemia, Hb H disease. The policy for action depends upon ethnic origin of the woman, as the risk of a patient of Indian, Pakistani or Afro-Caribbean origin being a carrier of α^0-thalassaemia is negligible.

a) African, Pakistani, Indian or **Middle East origin**
The chance of her being a carrier for α^0-thalassaemia is extremely low.
If the patient is not iron deficient, then the diagnosis is homozygous α^+-thalassaemia.
The NSC guidelines indicate that the partner need not be tested.

b) Mediterranean or Southeast Asian origin.
There is a significant chance of the patient being a carrier of α^0-thalassaemia.

Test partner.
If his MCH is < 25, and the results are consistent with α-thalassaemia, s*end maternal and paternal blood for DNA testing*.

c) British or North European origin

There is an extremely small chance of the patient being a carrier of α^0-thalassaemia. However the chance of the partner, if also of British origin, having α^0-thalassaemia trait is extremely small, and no hydrops fetus has ever been reported for such a couple.

The NSC guidelines indicate that the partner need not be tested.

***d)* Further studies for rare types of α^0-thalassaemia**

Ultrasound investigations: An assessment of the fetus in a pregnancy at possible risk for Hb Bart's Hydrops fetalis syndrome may be done by ultrasound. Fetuses thought to be at risk for these rare cases of Hb Bart's hydrops fetalis syndrome can be examined for signs of anaemia and hydrops fetalis. These women should be referred for assessment of fetal anaemia using middle cerebral artery doppler peak systolic velocities, which will become abnormal before clinically apparent hydrops. These investigations should be available at the regional fetal medicine unit.

5. Summary of Guidance Notes

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

Conclusion for maternal carrier state	Conclusion for paternal carrier state	Further studies by DNA analysis
Normal	Partner testing not required	None required
Any	normal	None required
Hb S	Hb S or Hb C	None required until PND
Hb S	Hb O-Arab, D-Punjab, Lepore, β-thalassaemia	Send bloods for mutation confirmation
Hb S	HPFH	Send bloods for mutation confirmation
Hb S + α-thalassaemia	As for Hb S	As for Hb S
Hb C	Hb S	None required until PND
Hb D	Hb S	Send bloods for mutation confirmation
Hb O Arab	Hb S , β-thalassaemia	Send bloods for mutation confirmation
Hb Lepore	Hb S, E, O-Arab, Lepore, β-thalassaemia	Send bloods for mutation confirmation
Hb E	β-thalassaemia, Hb Lepore, α-thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation
β-thalassaemia	Hb S, E, O-Arab, Lepore, β-thalassaemia	Send bloods for mutation confirmation
β-thalassaemia	α-thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation if Med or Southeast Asian origin

Conclusion for maternal carrier state	Conclusion for paternal carrier state	Further studies by DNA analysis
α-thalassaemia (MCH of 25-27pg) 1) Indian, Pakistani or African 2) Southeast Asian, Mediterranean or other origin	Partner testing not required Partner testing not required	None required None required
α-thalassaemia (MCH < 25pg) 1) Indian, Pakistani, African, or North European 2) Southeast Asian, East Mediterranean (Cyprus Greece or Turkey), Middle Eastern or other origin	Partner testing not required Test partner	None required Send bloods for mutation confirmation if partner has MCH <25pg

Appendix 1: Table of parental carrier state combinations that give rise to the risk of an affected fetus.

The main haemoglobinopathy carrier state combinations that can result in an affected pregnancy are summarised in the table below (courtesy of Prof. B. Modell):

Carrier of:	α+ thal	αo thal	Hb S	β thal	δβ thal	Hb Lepore	Hb E	Hb O Arab	Hb C	Hb D Punjab	HPFH	Not a carrier
α+ thal												
αo thal												
Hb S												
β thal												
δβ thal												
Hb Lepore												
Hb E												
Hb O Arab												
Hb C												
Hb D Punjab												
HPFH												
Not a carrier												

Key:

	Serious risk
	Less serious risk
	Possible hidden risk of α^o thal
	No risk

Appendix 2. References

Guidelines for the fetal diagnosis of globin gene disorders. Globin Gene Disorder Working Party of the BCSH General Haematology Task Force. J. Clin. Pathol. (1994) 47: 199-204.

Guidelines for the investigation of the α and β thalassaemia traits. The Thalassaemia Working Party of the BCSH General Haematology Task Force. J. Clin. Pathol. (1994) 47: 289-295

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Screening and genetic diagnosis of haemoglobin disorders. Old J. M. Blood Reviews (2003) 17; 43-53

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