National Haemoglobinopathy Reference Laboratory

Information for Users

Summary

The NHRL offers a service for the identification of haemoglobinopathy genotypes by the molecular analysis of DNA and haematological investigation. This includes the investigation of difficult/complex phenotypes and the identification of carrier states for antenatal patients. It also offers a prenatal diagnosis service by fetal DNA analysis for sickle cell disease, β-thalassaemia and α-thalassaemia.

DNA tests: There are 5 categories of molecular haemoglobinopathy investigations performed by the NHRL, described in detail in section 1 of this document. The five tests are:

1) α-thalassaemia mutation/s identification (all α^+ and α^0 types),
2) β-thalassaemia, mutation/s identification (all β, δβ-thalassaemia and HPFH type deletions)
3) Sickle cell disorders (all genotypes)
4) Hb variant identification (all α-chain and β-chain variants)
5) Prenatal diagnosis for α^o-thalassaemias, β-thalassaemias and sickle cell disorders

Costs: The charges for haemoglobinopathy testing are detailed on page 7. Tariffs are structured according to the complexity of the tests performed to identify and report the haemoglobinopathy mutations involved. The charging structure is based on the number of amplifications required according to the UKGTN /ACGS guidelines. Thus the costs of the tests for each of the five types of investigation are the same for each level of complexity (see Table on page 7).

Indications for sample referral: Guidelines for which samples should be sent for DNA analysis are listed in section 2. A more detailed document "Guideline for DNA referral" is available and can be downloaded

Sample referral details: Guidelines for the referral of blood samples for to the NHRL for either phenotype/genotype investigations or prenatal diagnosis are presented in section 3. A specific NHRL referral form for accompanying blood samples is available and can be downloaded.

Opening Times. The NHRL is staffed from 9.00 to 17.00 hours, Mon–Fri, except for bank holidays.

1) The Five Molecular Diagnostic Investigations

There are 5 investigations that can be requested on the referral forms. The molecular analyses included in each investigation are detailed below:

a) α-thalassaemia:

α-thalassaemia mutations in the α-globin genes are screened for as appropriate, using Gap-PCR, MLPA, RE-PCR and DNA sequencing. The α-thalassaemia investigation includes molecular analysis for:

- α^{+}-thalassaemia deletions: $-\alpha^{3.7}$, $-\alpha^{4.2}$
- α^{+}-thalassaemia: all non-deletion mutations
- α^{0}-thalassaemia: $--^{MED}$, $-(\alpha)^{20.5}$, $--^{SEA}$, $--^{THAI}$, $--^{FIL}$,
- α^{0}-thalassaemia: $--^{BRIT}$, $--^{SA}$, and all rare/novel deletion mutations

b) β-thalassaemia:

All known β-thalassaemia mutations, and all δβ-thalassaemia / HPFH deletion mutations are screened in a referral for β-thalassaemia mutation analysis, using ARMS-PCR, RE-PCR, Gap-PCR, DNA sequencing and MLPA as appropriate. The β-thalassaemia investigation includes:

- β-thalassaemia: all point mutations in all ethnic groups.
- δβ-thalassaemia, $^{A}\gamma\delta\beta$-thalassaemia and εγδβ-thalassaemia
- HPFH deletion genes
- Non deletional HPFH - γ-promoter mutations
- Fusion genes Hbs Lepore and Kenya
- Triple α-gene analysis in thalassaemia intermedia cases and patients with unusually severe phenotype for β-thalassaemia trait.
- Xmn1 and α-thalassaemia status of thalassaemia intermedia/major patients.
- Increased α-globin gene copy number by MLPA or Gap-PCR

c) Sickle cell disease:

Sickle cell disease genotypes are analysed by ARMS-PCR, RE-PCR, Gap-PCR, MLPA, DNA sequencing and pyrosequencing as appropriate. The sickle cell investigation includes

- Hb S/S, Hb S/Hb D-Punjab, Hb S/Hb O-Arab, Hb S/C,
- Hb S/β-thalassaemia, Hb S/δβ-thalassaemia, Hb S/HPFH.
- Xmn1 status of affected patients with high Hb F levels
- α-thalassaemia status of affected patients if appropriate

d) Hb Variant:

All haemoglobin variants are identified by DNA analysis. Referrals for diagnosis of an unknown variant are analysed first by HPLC and IEF, and then identified/confirmed by DNA analysis using ARMS-PCR, RE-PCR, Gap-PCR, MLPA, DNA sequencing, as appropriate. The Hb variant investigation includes:

- ***Confirmation of the clinically important Hb variants in antenatal patients:***
  The variants Hb D-Punjab, O-Arab and Lepore require confirmation by DNA analysis in all antenatal patients. The clinically important abnormal haemoglobins Hb S, C, E, are identified by DNA analysis only in couples requiring prenatal diagnosis.

- ***Identification of unknown / rare Hb variants in antenatal patients:*** These will be identified by DNA sequencing as a matter of urgency if partner is a haemoglobinopathy carrier.

- ***Identification of unknown rare Hb variants in non-antenatal patients***: These are characterised first by HPLC and IEF, and then identified/confirmed by DNA sequencing of the α1, α2 and β-globin genes.

e) Prenatal Diagnosis:

The prenatal diagnosis investigation involves the molecular determination of a fetal genotype and identification/confirmation of the maternal and paternal genotypes. To provide the safest possible result and in accordance with best practise guidelines, **two** different molecular diagnostic techniques are used to arrive at the result. The PND investigation also includes a check for maternal DNA contamination of the fetal DNA sample by a study of the inheritance pattern of 11 polymorphic STR markers from the mother and father.

Prenatal diagnosis is performed by mutation analysis of parental DNA (prepared from fresh blood samples whenever possible) and fetal DNA (prepared from chorionic villi, cultured chorionic villi, amniotic fluid, cultured amniocytes or fetal blood).

The genetic risks for prenatal diagnosis are detailed in our DNA referral guidelines document and also found in the "Handbook for Laboratories" published on-line by the NHS Sickle Cell and Thalassaemia Screening Programme as detailed below.

In summary, we carry out prenatal diagnosis for couples at risk of having a child affected with:

- Hb Bart's Hydrops fetalis (homozyous α^0-thalassaemia)
- Hb H hydrops fetalis involving severe non-deletion α^+-thalassaemia mutations
- homozyous β-thalassaemia,
- β-thalassaemia co-inherited with δβ-thalassaemia, Hb Lepore, Hb E, Hb O-Arab
- Sickle cell disorders: Hb S/S, S/C, S/D-Punjab, S/O-Arab, S/ β-thalassaemia

2) Samples Requiring DNA Investigation

Antenatal samples

Comprehensive guidelines for which patient samples require further investigation for antenatal screening purposes are contained in the "Handbook for Laboratories" published by the NHS Sickle Cell & Thalassaemia Screening Programme Laboratory Subgroup. The guidelines can be downloaded from the Screening Programme website http://sct.screening.nhs.uk/

Non-antenatal samples

The following haemoglobinopathies should be referred for genotyping by DNA studies:

- patients with Hb H disease,
- β-thalassaemia major & intermedia
- sickle β-thalassaemia, sickle HPFH
- Hb E / β-thalassaemia
- Hb E / α-thalassaemia
- Any other complex haemoglobinopathy

A definitive diagnosis of the following carrier states can only be made by DNA analysis. ***If in doubt about a referral please telephone the lab for advice.***

- α^0-thalassaemia & homozygous α^+-thalassaemia (in high risk groups)
- Hb D-Punjab
- Hb E
- unknown Hb variants,
- δβ-thalassaemia & HPFH trait,
- β-thalassaemia trait
- β-thalassaemia trait with borderline-raised Hb A_2 value (silent β-thalassaemia),
- patients with a split Hb A_2 value which add up to >3.5%
- Any other complex haemoglobinopathy

3) Sample Referral Procedures

Tests 1- 4: carrier state/genotyping request:

A fresh 10ml EDTA blood samples should be sent with:

a. a completed genotype referral request form with patient information clearly supplied,
b. haematological details of the patient (full blood count, Hb A_2 and F values, iron status, Hb electrophoresis results eg HPLC)

There are separate referral forms for genotype analysis and prenatal diagnosis. The referral form must be filled in and returned with the samples, together with the family origin form.

For optimal DNA analysis results, blood samples should be less than 5 days old. However samples up to one month old that have been kept refrigerated may give satisfactory results.

Samples should always be sent in appropriate packaging by first class post, or for urgent samples, by courier service.

Turn around times: these depend on the urgency of the sample and how many molecular investigations are required to identify the mutations. The target turnaround time for genotyping urgent antenatal patients is two weeks. For non-antenatal patients, the target turn around time is three to eight weeks, but a few very complex analyses may take longer.

Test 5: Prenatal diagnosis request:

The lab must be telephoned in advance to make arrangements for the referral of a prenatal diagnosis case, including provision of safe contact details to report the result by telephone and fax.

Requirements: Fresh parental blood samples must be sent with the fetal sample, together with a completed prenatal diagnosis request form with the following parent information clearly supplied: haematological details of both parents (full blood count, Hb A_2 and F values, Hb electrophoresis results.

Parental samples: Fresh 10ml EDTA blood samples from mother and father should be sent from all couples requiring prenatal diagnosis at the time of fetal sampling. This is required for control samples and for testing for maternal contamination, even if the mutation has been characterised previously. If the father is unavailable for blood sampling, a copy of his laboratory results stating his haemoglobin genotype should be provided. ***If the paternal genotype is unknown please contact the laboratory for advice.***

Fetal sample: CVS, amniotic fluid or fetal blood can be used to extract fetal DNA.

- **Chorionic villus biopsy sample (CVS):** The CVS **must** be cleaned by microscopic dissection to remove any contaminating maternal tissue before sending to the NHRL for DNA analysis. The referrer must arrange for this to be carried out at a local cytogenetics laboratory, and also instruct the lab to forward the sorted CVS by guaranteed post or courier to the NHRL with appropriate documentation.

It is recommended that a CVS culture is set up by the cytogenetics lab for back up purposes if this is not to be done routinely by the lab for karyotyping. The NHRL will contact the cytogenetic laboratory if the backup cultures are required.

The cleaned CVS should be sent to the NHRL in culture medium, saline or if possible, in CVS lysing solution (0.1-0.5ml depending upon size of the cleaned CVS sample). CVS lysing solution is 100mM NaCl / 25mM EDTA / 0.2% SDS / 0.4mg/ml Proteinase K.

- **Amniotic fluid sample:** Obstetric departments should aim to take approximately 20 mls of amniotic fluid. 10mls can then be forwarded directly to the NHRL for testing. The remaining 10mls can be sent to a local cytogenetics laboratory for back-up cultures. The NHRL will contact the cytogenetic laboratory if the backup cultures are required. If it is not possible to obtain 20mls of amniotic fluid please telephone the laboratory for advice.

- **Fetal blood:** On very rare occasions fetal blood sampling may be performed and a fetal blood sample sent in EDTA for analysis. For example, for the diagnosis of homozygous α^0-thalassaemia in a fetus diagnosed as hydropic by ultrasound.

Turn around times: The time taken for prenatal diagnosis is normally 3-5 working days upon receipt of fetal sample, provided the parental mutations are known beforehand.

Patient Consent:

Specific patient consent obtained for medical investigations for a haemoglobinopathy in one laboratory will permit the referral of the blood sample to another laboratory for: additional investigations of the haemoglobinopathy, the storage of the patient's DNA sample for any further investigations related to the patient's diagnosis in the future, the use of the patient's DNA for quality assurance in laboratory tests, and

the use of the patient's DNA for education and training of laboratory staff. It will not permit the analysis of the patient's DNA for any other genetic disorder without further specific consent for that test.

Each blood sample referred for DNA analysis should be accompanied by a referral form which has been signed by the requesting clinician/counsellor/nurse to state that patient consent for haemoglobinopathy DNA testing has been obtained.

Quality Assurance

The NHRL participates in the NEQAS "DNA diagnostics for haemoglobinopathies" pilot scheme, the NEQAS "Hb A_2/Hb F & abnormal haemoglobins" scheme, newborn sickle screening scheme and the NEQAS full blood count scheme.

Reference Ranges

Our reference ranges for haemoglobinopathy screening red cell indices are:

	***Hb** g/l*	***RBC** $10^6/mm^3$*	***MCV** fl*	***MCH** pg*	***Hb A_2** %*	***Hb F** %*
men	130-170	4.5-5.5	83-101	27-32	2.0-3.2	<1.0
women	120-150	3.8-4.8		27-32	2.0-3.2	<1.0

4) NHRL Tariffs:

The current tariffs for the diagnostic services provided by the NHRL are:

Band	***Charge £***	***Complexity of report***	***Type of report***
1	50	Basic	*Haematology only (includes FBC, HPLC, IEF)*: interpretation of results
2	100	Simple	*Haematology & DNA testing (1amplicon):* Gap-PCR for α-thalassaemia, confirmation of a known mutation in family members
3	250	Moderate	*Haematology & DNA testing (2-4 amplicons):* Genotyping by MLPA, ARMS-PCR, RE-PCR, etc for unknown mutations, sequencing of gene for a known mutation
4	500	Complex	*Haematology & DNA testing (5-19 amplicons):* Complex tests – e.g. sequencing/testing for unknown mutations in multiple genes
5	1150	PND[1]	*Haematology & DNA testing of fetal and parental DNA samples* Complex tests including test for maternal contamination

[1]Note - the prenatal diagnosis tariff includes the cost of the molecular determination of the fetal genotype in triplicate and the identification/confirmation of the maternal and paternal genotypes, by using **two** different molecular diagnostic techniques. Exclusion of maternal contamination by analysis of 11 STR polymorphic markers in the maternal, paternal and fetal DNA is also included.

The NHRL was centrally funded by the DH from 1982 to 31st March 2006, after which the central funding ceased and the DH required the NHRL to charge for its service on a provider to provider basis. Our central funding was devolved to all PCTs to pay for haemoglobinopathy DNA studies.

Invoicing

Invoices will be sent monthly from the Oxford University Hospitals Trust Finance Department to you, or the nominated contact provided by you on the referral form.

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