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. Haemoglobinopathy investigations are also undertaken as part of molecular testing for rare inherited anaemias.

Opening Times. The NHRL is staffed from 9.00 to 17.00 hours, Mon–Fri, except for bank holidays.
To contact the laboratory please phone 01865 572769 or email oxford.molecularhaem@nhs.net
Further information and referral forms are available on our website: https://www.oxford-translational-molecular-diagnostics.org.uk/

1) NHRL Tariffs and target turnaround times (TAT):

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

Charge £	***Type of report***	***Target TAT*** *(from sample receipt in lab)*
50	*Haematology only (includes FBC, HPLC, IEF where appropriate)*: interpretation of results	3 days for urgent neonates 2 weeks for other samples
250	*Haematology (as above) & DNA testing* DNA sequencing of alpha and beta globin genes, Gap-PCR for 3.7/4.2 alpha plus thalassaemia and further testing where deemed appropriate (alpha gene MLPA, beta globin cluster MLPA, sequencing of the gamma and delta globin genes)	42 calendar days for routine samples 21 calendar days for urgent antenatal or neonatal referrals
500	*Haematology & DNA testing for rare anaemia:* Haematology as above Haemoglobinopathy investigations as above Next generation sequencing of 51 additional genes associated with rarer genetic disorders involving anaemia (requires a different referral form)	84 calendar days for routine samples 42 days for single gene family tests 21 days for urgent antenatal or neonatal referrals
1150	*Prenatal diagnosis* *Haematology & DNA testing of fetus and parents* Predictive genetic testing of the fetus based on the parental genotypes. Includes parental genetics and haematology and test for maternal contamination	3 days where the mutations in both parents are known in advance Up to 21 days where the mutations are not known or if the father is not available for testing

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. However central funding will

soon (most likely 2019) be provided by NHSE for individuals living in England meeting the referral criteria (please see https://www.england.nhs.uk/publication/national-genomic-test-directories/)

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.

2) Sample Referral Procedures

Ideally fresh 3-4*ml EDTA blood sample labelled with the patient's forename, surname, date of birth and NHS number (if appropriate) should be sent with:

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

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.

*DNA extraction can be attempted from blood samples of 0.5-2ml but may not provide adequate amounts of DNA to complete complex analyses.

DNA extracted elsewhere is also accepted for genetic testing, but it is ESSENTIAL that the red cell indices and protein analysis (HPLC or similar) is provided for these samples to enable correlation of genetic results with the patient phenoptype.

There are separate referral forms for genotype analysis and prenatal diagnosis (available for download from our website: https://www.oxford-translational-molecular-diagnostics.org.uk/content/forms . The referral form must be filled in and returned with the samples.

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

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 email.

Requirements: Fresh parental EDTA 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).

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 alpha^0-thalassaemia in a fetus diagnosed as hydropic by ultrasound.

Patient Consent:

Patient consent should be obtained for obtained for medical investigations for a haemoglobinopathy/rare anaemia. This permits 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.

Quality Assurance
The NHRL participates in the NEQAS "DNA diagnostics for haemoglobinopathies" scheme, the NEQAS "Hb A_2/Hb F & abnormal haemoglobins" scheme, newborn sickle screening scheme and the NEQAS full blood count scheme. We also participate in the NEQAS schemes for maternal contamination, NGS panels and pathogenicity investigation.

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

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