

Short User Guide

Oxford BRC Haemato-Molecular Diagnostic Service

The Oxford University Hospitals NHS trust's Department of Laboratory Haematology provides a comprehensive molecular diagnostic service for a range of haematological conditions. The services offered are divided into 4 main areas:-

- 1) **Haemostasis:** Haemophilia and thrombophilia genetic testing
- 2) **Haemoglobinopathies:** A national service offering extensive molecular investigation of α -thalassaemia, β -thalalassaemia, abnormal haemoglobins, and the sickle cell syndromes.
- 3) **Haemachromatosis:** Screening for the HFE gene mutations
- 4) **Haemato-oncology:** An integrated phenotypic (immunophenotyping) and molecular service for the management of haematological malignancies.

This document is intended as a brief and provisional introduction to our services. More detailed information on all aspects of our service can be obtained from our web site (www.oxfordradcliffe.nhs.uk/molhaem) or requested by e-mail from <mailto:molhaem@ouh.nhs.uk>

General Information

Laboratory address for specimen reception	Molecular Haematology, Dept. of Laboratory Haematology, Level 4, John Radcliffe Hospital Oxford, OX3 9DU
CPA Accreditation	Accredited: Ref No. 1040
Lab service hours	9:00-5:00 Monday to Friday
Enquiries and information:- Website: E-mail for advice and enquiries:- Haematology molecular genetics laboratory Immunophenotyping laboratory Fax:	www.oxfordradcliffe.nhs.uk/molhaem molhaem@ouh.nhs.uk 01865 572769 01865 572827 01865 572775
Clinical and BRC Research leads Scientific Director Deputy Scientific Director Laboratory Manager	Dr Anna Schuh <i>MD, PhD, MRCP, FRCPath</i> Dr Chris Hatton <i>FRCP, FRCPath</i> Dr John Old <i>PhD, FRCPath</i> Dr Shirley Henderson <i>MSc PhD</i> Mr Dan Smith <i>C.Sci. FIBMS</i>

Request Form and Samples

All samples should be accompanied by a completed request form. This is available on our website. Specimens and forms should have a minimum of 4 patient identifiers including patient surname, first name, dob and hospital number.

Please provide as much clinical and laboratory information as possible, including a brief clinical history and any others recent results available on the patient. Indicate on the form the sample type, date and time of collection and the investigation that you are requesting. Please remember to give full contact details for results and reports.

The sample type required for each investigation is shown in the appropriate section below. All samples should be addressed to Molecular Haematology and sent to the specimen reception of the Haematology Laboratory at the John Radcliffe Hospital, Level 4. Address is given in general information (page 1).

Investigations Offered

1) Haemostasis

Disorder	Tests
Haemophilia A	Factor VIII gene intron 22 inversion (inverse PCR) Factor VIII gene intron 1 inversion (PCR) Detection of Factor VIII mutations by direct sequencing
Haemophilia B	Identification of Factor IX mutations by direct sequencing
VWD	Detection of known VWF gene mutations by direct sequencing Targeted VWF gene screening Type 2A, 2B, 2N and 2M by direct sequencing Gene linkage analysis (intron 40 VNTR s)
Other Disorders	Factors V, VII, FXIII and FX by direct sequencing
Thrombophilia	Factor V Leiden and prothrombin 20210 mutations by multiplex PCR

Specimens required: 10ml EDTA peripheral blood

Turnaround times: Haemophilia's A & B and VWD: 2- 8 weeks depending on complexity
Thrombophilia genetic screening: 2 weeks (full thrombophilia screening takes longer)
Prenatal Diagnosis*: 3-5 working days

**Prenatal diagnosis of haemophilia by DNA analysis available by prior arrangement with the laboratory.*

2) Haemoglobinopathies

This service is provided by the National Haemoglobinopathy Reference Laboratory which provides a tertiary referral service for all hospitals throughout the UK, Ireland and abroad. Genetic tests for all known haemoglobinopathy mutations are available.

Disorder	Tests
α -thalassaemia	Detection of deletions using Gap-PCR and MLPA. Detection of non-deletion α^+ mutations by α -globin gene sequencing.
β -thalassaemia	Genotyping using ARMS-PCR, Restriction Enzyme-PCR, Gap-PCR, MLPA or DNA sequencing.
HPFH and $\delta\beta$ -thalassaemia	Detection of deletions by Gap-PCR, and MLPA.
Sickle cell disease:	Genotyping by ARMS-PCR, Restriction Enzyme-PCR, Gap-PCR, MLPA or DNA sequencing.
Hb Variants	Identification by ARMS testing or α and β -globin gene sequencing.

Specimen required: 10ml EDTA peripheral blood

Turnaround time: 2- 8 weeks depending on the complexity of the investigation

Prenatal diagnosis*: 3-5 working days

*Prenatal Diagnosis of sickle cell disease, β -thalassaemia and Hb H/Bart's hydrops fetalis available by prior arrangement with the laboratory

3) Haemochromatosis (HC)

Indications: family history of iron overload, unexplained high ferritin

Indications	Test
On all patients with suspected HC	HFE gene mutation analysis: C282Y/H63D
If HFE gene mutation negative, but high ferritin or transferrin saturation and positive family history of HFE mutation negative HC	Haemojuvelin, hepcidin and ferroportin gene mutation analysis
Asian patients with suspected HC	Haemojuvelin mutation analysis

Specimen required: 5ml EDTA peripheral blood

Turnaround time: Common mutations: - 2 weeks

Rare mutations/complex cases: 8 weeks

4) Haemato-Oncology**a) Molecular Genetics / minimum residual disease monitoring**

Molecular genetic testing uses PCR (DNA) and RT-PCR (RNA) methodologies to detect common chromosomal abnormalities of clinical, diagnostic or prognostic significance in malignant haematological conditions.

Disorder	Test	Type
Acute lymphoblastic leukaemia	BCR-ABL t(9;22) by multiplex PCR	RNA
Acute Myeloid Leukaemia	NPM1, FLT3 ITD and D835TK, CEBPA PML-RARA t(15;17)	DNA RNA
Lymphoma: B-cell clonality	IgH FR1, FR2, FR3 re-arrangements IgH incomplete D-J re-arrangements using BIOMED 2 CE marked primers	DNA
Lymphoma: T-cell clonality	TCRB and TCRG and TCRD gene rearrangements using BIOMED 2 CE marked primers	DNA
Chronic lymphocytic leukaemia	Somatic hypermutation analysis using leader and biomed 2 primers TP53 mutation analysis by FISH and HRM	DNA/Blood slide
Chronic myeloid leukaemia	BCR-ABL t(9;22) by multiplex PCR and quantitative PCR	RNA
Myeloproliferative disorders	JAK2 V617F mutation by allele specific PCR and pyrosequencing, Exon 12 and MPL mutation analysis BCR-ABL by multiplex PCR	DNA RNA
Haemopoietic Stem Cell Transplantation	STR-Chimerism (total white cell, CD3, CD19, CD56 and CD34 positive cells).	DNA
Non Hodgkin's lymphomas (NHL)	FISH studies for chromosomal rearrangements t(8;14); (14;18); (11;14); (11;18); (2;5); del17p13.1, 11q22.3 Performed only after histopathology review	FFPE Slides
Myeloma	FISH studies for TP53 deletions and chromosome 4:14 translocation.	DNA/Blood slides

Specimen required DNA based tests: 5ml of EDTA peripheral blood or bone marrow
RNA based tests: 20ml of EDTA peripheral blood or 2ml of bone marrow; must arrive in lab within 36 hours of collection.

Turnaround time: Urgent samples (all acute leukaemia diagnostics):-

- fusion genes – 3 working days
- NPM1, FLT3 ITD and D835 TK – 1 week
- JAK2, Clonality studies, STR Chimerism and BCR-ABL:- 2 weeks
- Somatic hypermutation:- 8 weeks

b) Immunophenotyping

Immunophenotyping is performed by flow cytometry on a six channel Becton Dickinson. The following antibody panels are available:

Disorder	Tests
Acute leukaemia	T cell antibodies (CD2, CD3, CD7) B cell antibodies (CD10, CD19, CD79a, cytoplasmic μ) Myeloid antibodies (CD13, CD14, CD33, CD64, CD117, MPO) Others (CD34, CD56, HLA-DR, TdT)
B-lymphoproliferative	CD3, CD5, CD10, CD19, CD20, CD23, CD38, CD79b, kappa, lambda
T/NK lymphoproliferative	CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD19, CD56, kappa, lambda
Hairy cell leukaemia	B-lymphoproliferative panel, CD11c, CD22, CD25, CD103
Multiple myeloma	CD19, CD38, CD45, CD56, CD138, kappa, lambda
PNH	CD15, CD16, CD55, CD59

Specimen required: Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml).
Unstained blood/bone marrow smears.

Turnaround time: Urgent samples are processed within 24 hours and results are communicated by e-mail and telephone. All results are discussed at the MDT meetings and authorised weekly.



Accredited Medical Laboratory
Reference No: 1040

