OXFORD Molecular Diagnostics Centre

Oxford University Hospitals NHS Trust

Short User Guide

Oxford BRC Haemato-Molecular Diagnostic Service

The Oxford University Hospitals NHS trust's department of 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) Iron Regulation: Screening for the HFE gene mutations

4) Haemato-oncology: An integrated phenotypic (immunophenotyping) and molecular service for the management of haematological malignancies.

5) Solid tumours: Integrated pathology and genomics. CE marked diagnostics of response prediction and cancer gene mutation panel utilising next generation sequencing.

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 (http://www.oxford-translational-molecular-diagnostics.org.uk/) or requested by e-mail from oxford.molecularhaem@nhs.net.

General Information

Laboratory address for specimen reception	Molecular Haematology, Level 4, John Radcliffe Hospital, Headington Oxford, OX3 9DU
CPA Accreditation	Accredited: Ref No. 1040
Lab service hours	9:00-5:00 Monday to Friday
Enquiries and information:- Website:	http://www.oxford-translational-molecular-diagnostics.org.uk/

UNIVERSITY OF OXFORD

Oxford Biomedical Research Centre

NHS National Institute for Health Research

CPA Accredited Medical Laboratory Reference No: 1040

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Clinical and BRC Research leads **Scientific Director** **Business Manager** **Haematology Laboratory Manager**	Dr Anna Schuh *MD, PhD, MRCP, FRCPath* Dr Chris Hatton *FRCP, FRCPath* Dr Shirley Henderson *MSc PhD* Dr Nick Housby *PhD* Mr Dan Smith *C.Sci. FIBMS*

Request Form and Samples

All samples should be accompanied by a completed request form (page 6). For haemoglobinopathy investigations, a more detailed NHRL request form is available from our web site. 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 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	Specimen Required	Turnaround Time
Haemophilia A Haemophilia A	*F8* gene intron 22 inversion (inverse PCR) *F8* gene intron 1 inversion (inverse PCR) *F8* mutations by direct sequencing Carrier analysis and full genetic screen Dosage analysis (MLPA) for partial/complete *F8* gene deletions/duplications	4ml EDTA peripheral blood 4ml EDTA peripheral blood	2-8 weeks depending on complexity 2-8 weeks depending on complexity
Haemophilia B	*F9* mutations by direct sequencing	4ml EDTA	2-8 weeks

Disorder	Tests	Specimen Required	Turnaround Time
	Carrier analysis and full genetic screen. Dosage analysis (MLPA) for partial/complete *F9* gene deletions/duplications	peripheral blood	depending on complexity
VWD	Detection of known *VWF* gene mutations by direct sequencing Targeted *VWF* gene screening for Type 2A, 2B, 2N and 2M by direct sequencing Full genetic screening for Type 1 and Type 3 VWD Dosage analysis (MLPA) for partial/complete *VWF* gene deletions/duplications	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Other Disorders	*F5*, *F7*, *F10*, *F11*, *F13A* by direct sequencing. Fibrinogenaemias (α, β and γ genes) Antithrombin deficiency (*SERPINC1* gene) Carrier and full gene analysis Dosage analysis (MLPA) for partial/complete *F7* and *SERPINC1* gene deletions/duplications	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Platelet Disorders	May-Hegglin anomaly (*MYH9* gene). Glanzmann Thrombasthenia (*ITGA2B & ITGB3* genes) Bernard-Soulier syndrome (*GpIbα , Gp9* and *GpIbb* genes) Platelet-type pseudo VWD (*GpIbα* gene)	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Thrombophilia	Factor V Leiden and prothrombin 20210 mutations by multiplex PCR	4ml EDTA peripheral blood	2 weeks

***Prenatal diagnosis of haemophilia by DNA analysis available by prior arrangement with the laboratory**. Turnaround time 3-5 working days.*

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	Specimen Required	Turnaround Time
α-thalassaemia	Detection of deletions using Gap-PCR and MLPA. Detection of non-deletion α^+ mutations by α-globin gene sanger sequencing.	2*4ml EDTA peripheral blood	2-6 weeks depending on complexity
β-thalassaemia	Detection of non-deletion mutations using sanger sequencing, pyrosequencing and ARMS-PCR. Detection of deletions using MLPA and Gap-PCR.	2*4ml EDTA peripheral blood	2-6 weeks depending on complexity
HPFH and δβ-thalassaemia	Detection of deletions by MLPA and Gap-PCR. Detection of non-deletion HPFH using sanger sequencing of the gamma gene promoters.	2*4ml EDTA peripheral blood	2-6 weeks depending on complexity
Sickle cell disease	Genotyping by sanger sequencing, pyrosequencing and ARMS-PCR.	2*4ml EDTA peripheral blood	2-6 weeks depending on complexity
Hb Variants	Identification by sanger sequencing of the α, β, γ and δ-globin genes.	2*4ml EDTA peripheral blood	2-6 weeks depending on complexity

***Prenatal Diagnosis** of sickle cell disease, β-thalassaemia and Hb H/Bart's hydrops fetalis available by prior arrangement with the laboratory. Turnaround time 3-5 working days.*

3) Iron Regulation

Indications: family history of iron overload, unexplained high ferritin.

Indications	Test	Specimen Required	Turnaround Time
On all patients with suspected Haemochromatosis.	HFE gene mutation analysis: C282Y/H63D	4ml EDTA peripheral blood	2 weeks
Hyperferritinaemia with normal serum iron and Transferrin saturation. Hereditary Hyperferritinaemia cataract syndrome	*SLC40A1* gene (Ferroportin) *IRE 5'UTR of FTL* gene. FTL gene. Carrier and full genetic screening by sequencing.	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Patients with iron overload, lethargy, liver disease, cardiomyopathy, diabetes, endocrine problems, arthritis, abdominal pain, skin pigmentation. Juvenile haemochromatosis: Severe iron overload, diabetes, cardiomyopathy, endocrine problems, hypogonadotrophic hypogonadism	*HFE2*, *HAMP*, *SLC40A1*, *TFR2* and *HFE* gene mutation analysis. Dosage analysis (MLPA) for partial/complete *HFE*, *HFE2*, *HAMP*, *TFR2* and *SLC40A1* gene deletions/duplications	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Asian patients with suspected HC Severe iron overload, diabetes, infertility, endocrine problems, hypogonadotrophic hypogonadism	*HFE2* and *HAMP* mutation analysis first, then the rest of the genes Dosage analysis (MLPA) for partial/complete *HFE*, *HFE2*, *HAMP*, *TFR2* and *SLC40A1* gene deletions/duplications	4ml EDTA peripheral blood	2-8 weeks depending on complexity
Iron overload (negative for the 2 common North European mutations) Non Caucasians with unexplained iron overload	NGS TSCA Iron Regulatory Gene Panel is based on the following gene sets:- *TFR2*, *SLC40A1*, *HFE*, *HFE2*, *HAMP*, *TF*, *FTL*, IRE of *FTL*, *SLC11A2*, *TMPRSS6*, *HEPH*, *FTH1*, *CP*, ALAS2, *BMP4*, *BMP6*, *SMAD4*.	4ml EDTA peripheral blood	8 weeks

Indications	Test	Specimen Required	Turnaround Time
Iron regulatory iron deficiency anaemia (IRIDA), unexplained anaemia Hyperferritinaemia Hereditary Hyperferritinaemia Cataract Syndrome (HHCS) Atransferrinaemia Aceruloplasminaemia Hereditary ferritinopathy X-linked Sideroblastic anaemia	NGS TSCA Iron Regulatory Gene Panel is based on the following gene sets:- *TFR2*, *SLC40A1*, *HFE*, *HFE2*, *HAMP*, *TF*, *FTL*, IRE of *FTL*, *SLC11A2*, *TMPRSS6*, *HEPH*, *FTH1*, *CP*, ALAS2, *BMP4*, *BMP6*, *SMAD4*.	4ml EDTA peripheral blood	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	Sample Type	Specimen Required	Turnaround Time
Acute lymphoblastic leukaemia	BCR-ABL t(9;22) by multiplex PCR and quantitative PCR	RNA*	20ml of EDTA peripheral blood or 2ml of bone marrow	3-5 working days
Acute Myeloid Leukaemia	NPM1, FLT3 ITD and D835TK	DNA	4ml of EDTA peripheral blood or bone marrow	2 weeks

Disorder	Test	Sample Type	Specimen Required	Turnaround Time
Acute Myeloid Leukaemia	PML-RARA t(15;17), CBFB-MYH11 type A, RUNX1/RUNXT1	RNA*	20ml of EDTA peripheral blood or 2ml of bone marrow	3-5 working days
Lymphoma: B-cell clonality	IgH FR1, FR2, FR3 rearrangements Igk Vk-Jk, Vk-Kde + intron-Kde rearrangements IgL rearrangements using BIOMED 2 CE marked primers IgH incomplete D-J rearrangements using BIOMED primers	DNA	Minimum *5 FFPE Rolled Sections 4ml of EDTA peripheral blood or bone marrow	2 weeks
Lymphoma: T-cell clonality	TCRB and TCRG and TCRD gene rearrangements using BIOMED 2 CE marked primers	DNA	Minimum *5 FFPE Rolled Sections 4ml of EDTA peripheral blood or bone marrow	2 weeks
Chronic lymphocytic leukaemia	Somatic hypermutation analysis using leader and biomed 2 primers TP53 mutation analysis by FISH and sanger sequencing	DNA Blood or BM slide, DNA	4ml of EDTA peripheral blood or bone marrow	4 weeks
Chronic myeloid leukaemia	BCR-ABL t(9;22) by multiplex PCR and quantitative PCR	RNA*	20ml of EDTA peripheral blood or 2ml of bone marrow	2 weeks

Disorder	Test	Sample Type	Specimen Required	Turnaround Time
Myeloproliferative disorders	JAK2 V617F mutation by allele specific PCR and pyrosequencing	DNA	4ml of EDTA peripheral blood or bone marrow	2 weeks
	JAK2 Exon 12 and MPL W515 mutation analysis	DNA	4ml of EDTA peripheral blood or bone marrow	4 weeks
	BCR-ABL by multiplex PCR	RNA*	20ml of EDTA peripheral blood or 2ml of bone marrow	2 weeks
Haemopoietic Stem Cell Transplantation	SNP-Chimerism (total white cell, CD3, and CD34 positive cells).	DNA BM/PB	4ml of EDTA peripheral blood or bone marrow	2 weeks
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 BM/PB	FFPE Slide 4ml of EDTA peripheral blood or bone marrow	2 weeks

** Must arrive in lab within 36 hours of collection.*

b) ***Immunophenotyping***

Immunophenotyping is performed on a six channel Becton Flow Cytometer. The following antibody panels are available:

Disorder	Tests	Specimen Required	Turnaround Time
Acute leukaemia	T cell antibodies (CD2, CD3, CD7) B cell antibodies (CD10, CD19, CD79a, cytoplasmic □□□□gM) Myeloid antibodies (CD13, CD14, CD33, CD64, CD117, MPO, CD11) Others (CD34, CD56, HLA-DR, TdT, CD41, NGZ, Glycophorin A)	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	Usually processed within 24 hours*
B-lymphoproliferative	CD3, CD5, CD10, CD19, CD20, CD23, CD38, CD79b, kappa, lambda, FMC7	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	Usually processed within 24 hours*
T/NK lymphoproliferative	CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD19. CD56, kappa, lambda, CD57, TCR, CD25, α, β, γ, δ	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	Usually processed within 24 hours*
Hairy cell leukaemia	B-lymphoproliferative panel, CD11c, CD22, CD25, CD103	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	Usually processed within 24 hours*
Multiple myeloma	CD19, CD38, CD45, CD56, CD138, kappa, lambda	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural	Usually processed within 24 hours*

Disorder	Tests	Specimen Required	Turnaround Time
		Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	
PNH	FLAER, CD14, CD24, CD59	Blood (10ml EDTA) and/or Bone marrow (1-2 ml EDTA), CSF and Pleural Fluid (5-10 ml), as appropriate. Please note: blood/bone marrow smears should be unstained.	Usually processed within 24 hours*

**Results are communicated by e-mail and telephone. All results are discussed at the MDT meetings and authorised weekly.*

5) Solid Tumours

Identification of clinically actionable mutations utilising the COBAS system or tumour profiling using a clinically validated NGS based Cancer Panel.

Disorder	Tests	Specimen Required[#]	Turnaround Time
Colorectal Cancer	KRAS, BRAF, NRAS 50 gene NGS cancer panel	10 x 5um sections mounted on unstained slides	5 working days
Lung Cancer	EGFR, KRAS, EML4-ALK 50 gene NGS cancer panel	10 x 5um sections mounted on unstained slides. For EML4-ALK, 3x 5um unstained sections on coated slides,	5 working days
Melanoma	BRAF, NRAS 50 gene NGS cancer panel	10 x 5um sections mounted on unstained slides	5 working days
Other Cancer	50 gene NGS cancer panel*	10 x 5um sections mounted on unstained slides	10 working days

[#] *Specimen requirements - 10 x 5um sections mounted on unstained slides (or 5 if marked neoplastic area >2cm$^{)}$. Multiple sections can be placed on a single side. Please clean microtome blade and water-bath thoroughly before cutting sections to avoid cross-contamination and false positive results. Please include a H&E stained section from same block with tumour boundary marked. Tissue in this ring should be >70% neoplastic. Cytological material can be sent as for tissue blocks or send maximum available material (smears, touch preps etc.) on slides.*

*Genes included in the 50 gene panel:-

BRAF FGFR1 CTNNB1 SMO JAK3 EZH2 KRAS ERBB2 CDKN2A SMAD4 AKT1 GNA11 NRAS MET ABL VHL KDR GNAQ PDGFRA FGFR3 NOTCH1 NPM1 ALK IDH2 PIK3CA FLT3 ATM MPL JAK2 SRC KIT RB1 ERBB4 GNAS MLH1 APC PTEN CSF1R FGFR2 HNF1A HRAS CDH1 EGFR RET STK11 FBXW7 TP53 SMARCB1 PTPN11 IDH1

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