Oncology - Tests Performed According to Disease Entity

The oncology section provides genomic analysis of samples from patients with a range of haematological malignancies and lymphoproliferative conditions. The results obtained assist in diagnosis and classification, provide prognostic information for use in risk stratification, can direct therapeutic choice, and enable assessment of residual disease status post-treatment and/or post-transplant. The oncology section also provides FISH testing for a limited range of sarcomas and solid tumours.

The table below aims to provide information about available genomic testing according to clinical indication. Testing strategies may vary depending on sample type, sample volume, disease stage and specific requests by clinicians, therefore not all listed tests for a specific indication may be undertaken on every sample received.

The laboratory will attempt to preserve material for additional testing on all samples received. Specimens are retained by the laboratory in order to repeat analysis or to enable additional analysis to be performed. All samples are retained for a minimum of 5 years.
NGS. Next Generation Sequencing

Suspected diagnosis	Sample type	Cytogenetics (Test Directory code when applicable)	Molecular Genetics (Test Directory code when applicable)
Leucocytosis/Raised WBC/ Neutrophilia			
	PB	• *BCR/ABL1* FISH (M85.24) • Karyotype (on request or if *BCR/ABL1* positive by FISH)	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]
	BM	• *BCR/ABL1* FISH (M85.24) • Karyotype (M85.3)	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]
Eosinophilia			
	PB	*FIP1L1/PDGFRA* FISH (M85.7)	• Myeloid NGS panel (M85.2) [2] • *FIP1L1/PDGFRA* RT-PCR (M85.7) [5] • *STAT5B* variant testing [5,6] • *JAK2* exon 13 variant testing [5,6]
	BM	• *FIP1L1/PDGFRA* FISH (M85.7) • Karyotype (M85.3)	• Myeloid NGS panel (M85.2) [2] • *FIP1L1/PDGFRA* RT-PCR (M85.7) [5]

			• *STAT5B* variant testing [5,6] • *JAK2* exon 13 variant testing [5,6]
Erythrocytosis/Polycythaemia/Raised haematocrit/Thrombocytosis/Raised platelets			
	PB	FISH for *BCR/ABL1* if there is suspicion of CML (i.e. basophilia & thrombocytosis) (M85.24)	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]
	BM	• FISH for *BCR/ABL1* if there is suspicion of CML (i.e. basophilia & thrombocytosis) (M85.24) • Karyotype (on request; ?for triple negative) (M85.3)	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]
Myeloproliferative neoplasms (MPN) including: ET, PRV, MF			
Cases referred for suspected/confirmed MPN will be tested with an MPN-panel which uses targeted amplicon NGS to analyse the hotspots on the three candidate MPN genes *JAK2*, *CALR* and *MPL*. The assay provides information on all 3 genes simultaneously rather than requiring sequential testing. Chromosome analysis is not routinely performed for MPN due to the low abnormality rates, and will not be attempted on blood. However, this can be requested on specific cases for BM samples. The myeloid NGS panel is only performed when specifically requested.	PB	Tests available only <u>on request</u>: o FISH for *FIP1L1/PDGFRA* if there is eosinophilia. o FISH for *BCR/ABL1* if there is a strong suspicion of CML (e.g. basophilia with thrombocytosis) o Even if *JAK2/CALR/MPL* variants detected, some cases may also have *BCR/ABL1*	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]
	BM	Karyotype (on request; e.g MF; triple-negative cases) (M85.3) <u>Additional tests available on request</u>: o FISH for *FIP1L1/PDGFRA* if there is eosinophilia. o FISH for *BCR/ABL1* if there is a strong suspicion of CML (e.g. basophilia with thrombocytosis) o Even if *JAK2/CALR/MPL* variants detected, some cases may also have *BCR/ABL1*	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2]

Aplastic anaemia (AA)/MDS			
	PB	FISH (on request; minimal FISH panel: -5/5q- , -7/7q- & -17/17p-, *MECOM* b/a)	Myeloid NGS panel (M82.1) [2]
	BM	• Karyotype (M82.2) • FISH (if required, particularly if karyotype fails or limited analysis; minimal FISH panel: -5/5q- , -7/7q- & -17/17p-, *MECOM*/3q b/a)	Myeloid NGS panel (M82.1) [2]
MPN/MDS overlap syndromes			
	PB	FISH (on request; minimal FISH panel: -5/5q- , -7/7q- & -17/17p-, *MECOM*/3q b/a)	Myeloid NGS panel (M224.1) [2]
	BM	• Karyotype (M224.2) • FISH (if required, particularly if karyotype fails or limited analysis; minimal FISH panel: -5/5q- , -7/7q- & -17/17p-, *MECOM*/3q b/a)	Myeloid NGS panel (M224.1) [2]
Chronic Myeloid Leukaemia (CML) including CP, AP and BC			
Diagnosis. Urgent *BCR/ABL1* FISH, karyotype and RT-PCR to determine transcript breakpoints. **Post-treatment** bone marrows can be screened for Ph by cytogenetics or FISH to monitor the initial response to treatment. However, more sensitive methods would be required (such as real time quantitative RT-PCR) to monitor patients after cytogenetic remission is achieved. **Progression/transformation/rising level of BCR-ABL1 transcripts**. Karyotype and FISH. **Molecular monitoring**: molecular monitoring of the *BCR/ABL1* transcript using real-time quantitative RT-PCR is not performed at this laboratory, but at the Molecular Pathology	PB & BM	• FISH for *BCR/ABL1* (M84.3) • Karyotype to confirm t(9;22) and check for further abnormalities (M84.4) • Additional FISH to clarify/exclude additional abnormalities (when required)	*BCR/ABL1* RT-PCR (M84.1) [3]

Department at University Hospital Southampton. **<u>Testing for *BCR-ABL1* TKD mutations</u>** for patients who either fail to respond to treatment or lose their response is performed at the West Midlands Regional Genetics Laboratory in Birmingham. Samples for this test should be sent to the Molecular Pathology Department at University Hospital Southampton which will then forward material to the Birmingham Laboratory.			
Mast cell disorders/ mastocytosis			
	PB	X	• *KIT* D816 by digital PCR (M86.2) • Extended *KIT* NGS panel (exons 2, 8, 9, 10, 11 & 17) (M86.1) [8] • Myeloid NGS panel for SM-AHN (M85.2) [2]
	BM (preferable)	Karyotype only for advanced systemic mastocytosis on request (M85.3)	• *KIT* D816 by digital PCR (M86.2) • Extended *KIT* NGS panel (exons 2, 8, 9, 10, 11 & 17) (M86.1) [8] • Myeloid NGS panel for SM-AHN (M85.2) [2] • For patient <18 years: additional automatic testing for *KIT* K509I and *KIT* D419del [5]
Acute Myeloid Leukemia (AML)			
<u>Diagnosis</u>: All samples referred for AML or a high degree of suspicion of AML are activated for rapid *FLT3*-ITD and *NPM1* fragment analysis, G-band chromosome analysis and NGS analysis of a panel of relevant genes. Reflex FISH or further molecular testing may also be instigated to further characterise initial findings.	PB	• Karyotype depending on percentage of blasts and whether separate BM is available or not; if no BM received then PB will be processed for cytogenetics analysis • FISH if appropriate (e.g. *PML/RARA* for ?APL)	Only analysed if BM not available (see testes listed for BM below)
	BM	• Karyotype (M80.3) • FISH if appropriate (e.g. *PML/RARA* for ?APL)	• *FLT3*-ITD fragment analysis (M80.18) [7] • *NPM1* exon 12 fragment analysis (M80.22) [7] • *FLT3*-TKD targeted NGS panel (M80.21) [8] • *IDH1* & *IDH2* NGS targeted NGS panel (M80.23 & M80.24) [8]

Follow up: appropriate testing will be undertaken depending on diagnostic findings. **Measurable residual disease (MRD)** analysis by RTqPCR (when suitable molecular markers are available) is not performed at this laboratory. Samples for MRD testing will need to be sent to the Department of Molecular Pathology at University Hospital Southampton, where cDNA will be made and forwarded to the appropriate testing centre. **Relapse:** Testing as per initial presentation to identify clonal evolution and potential therapeutic targets. **Whole Genome Sequencing (WGS)**: is available for newly presenting AML patients on completion of appropriate documentation and submission of a germline sample (M80.1).			• *TP53* targeted NGS panel [8] • Myeloid NGS panel (M80.2) [2] • *CEBPA* Sanger seq • RT-PCR with Haemavision screen kit (M80.7) [4] • For Core Binding Factor AML, extended *KIT* NGS panel (exons 2, 8, 9, 10, 11 & 17) [8]
Acute Lymphoblastic Leukaemia (ALL) B- and T-cell			
Diagnosis: Full karyotype at diagnosis and rapid FISH for *BCR/ABL1*, *KMT2A* gene rearrangements, *ETV6/RUNX1* and iAMP21. Additional FISH for other markers (e.g. 'Ph-like', hyperdiploidy and hypodiploidy), as required. **Post-treatment:** screened for previous abnormality by FISH. **Relapse**. Full karyotype and relevant FISH	PB & BM	Analysis of PB depending on percentage of blasts and whether separate BM available or not; if no BM received then PB will be processed for cytogenetics/FISH analysis • Rapid FISH for *BCR/ABL1*, *KMT2A* (*MLL*), *ETV6/RUNX1* • Karyotype (M91.2) • Additional FISH panel as required (e.g. "Ph-like" FISH) • SNP array	RT-PCR with Haemavision screen kit (M80.7) [4]

SNP array is now performed on all pediatric cases in addition to G-banding analysis. This test is currently undertaken at the West Midlands Regional Genetics Laboratory in Birmingham. DNA from this test is extracted from the diagnostic sample which is standardly sent to the Birmingham laboratory for Minimal Residual Disease (MRD) monitoring. **MRD monitoring** using Rearrangement of T-Cell Receptor and Immunoglobulin H Genes is not performed at this laboratory. **Whole Genome Sequencing (WGS)**: is available for newly presenting ALL patients on completion of appropriate documentation and submission of a germline sample (M91.1).			
Chronic Lymphocytic Leukaemia (CLL)/Small Lymphocytic Leukaemia (SLL)			
CLL patients approaching first line of treatment: patients are tested for both *TP53* deletion by FISH and *TP53* mutation by NGS analysis. FISH is also routinely performed for 11q (*ATM*) deletion, del(13q) and trisomy 12. **IGHV hypermutation studies** are not performed at this laboratory, but at the Department of Molecular Pathology at University Hospital Southampton. (DNA may be forwarded on request is no separate sample has been sent).	PB & BM (BM not usually required for diagnosis)	FISH for: o *TP53* deletion (M94.4) o *ATM* (11q) deletion (M94.8) o Trisomy 12 (M94.10) o 13q14 deletion (M94.9) If Richter's suspected: o *MYC* gene rearrangement	*TP53* targeted NGS panel (M94.7) [8,9]

CLL patients approaching subsequent lines of treatment: Re-assessment of *TP53* status before each new line of therapy by both FISH and NGS panel analysis. **CLL patients on ibrutinib**: Some patients may become resistant to ibrutinib as a result of mutations in *BTK* and *PLCG2*. This test is not currently available at this laboratory; however, the West Midlands Regional Genetics Laboratory in Birmingham offers this service (DNA may be forwarded on request is no separate sample has been sent).			
Multiple Myeloma (MM)/ Plasma Cell Leukaemia (PCL)/AL Amyloidosis/MGUS			
	• BM • PB only for Plasma Cell Leukaemia	FISH: on CD138 separated cells for: o *IGH* gene rearrangement (M92.8) o *IGH/FGFR3* [t(4;14)] (M92.2) o *IGH/CCND1* [t(11;14)] (M92.4) o *IGH/MAF* [t(14;16)] (M92.5) o *IGH/MAFB* [t(14;20)] (M92.6) o *TP53/CEP17* (M92.12) o *CDKN2C* (1p32.3)/*CKS1B* (1q21) (M92.10 & M92.11) Upon request: o *MYC* gene rearrangement (M92.14)	Upon request: *TP53* targeted NGS panel on DNA extracted from CD138 separated cells [8,9]
Plasmablastic Lymphoma (PBL)			
	• BM • Formalin-fixed, paraffin-embedded tissue (FFPE) sections • Touch preps • Other sample type as required	Multiple myeloma FISH panel + FISH for: o *MYC* gene rearrangement o *IGH/MYC* [t(8;14)]	X

MALT lymphoma			
	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for: o *MALT* gene rearrangement (M107.4) o *BIRC3/MALT1* [t(11;18)] (M107.1) o *IGH/MALT1* [t(14;18)] (M107.3)	X
Mantle Cell Lymphoma (MCL)			
	• FFPE sections • Touch preps • PB/BM • Other sample type as required and infiltration confirmed	FISH for: o *IGH/CCND1* [t(11;14)] (M102.1) o *CCND2* gene rearrangement (M102.3) o *IGH/CCND3* [t(6;14)] o *TP53/CEP17* (on request)	*TP53* targeted NGS panel (M102.5) [8,9]
Follicular Lymphoma (FL)			
	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for: o *BCL6* gene rearrangement (M103.2) o *BCL2* gene rearrangement (M103.3)	X
High grade B cell lymphoma/DLBCL			
	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for: o *MYC* gene rearrangement (M99.1) o *IGH/MYC* [t(8;14)] (M99.2) If *MYC* rearranged: o *BCL2* gene rearrangement (M99.5) o *BCL6* gene rearrangement (M99.7) If *MYC* rearranged but not with *IGH*: o *IGK/MYC* [t(2;8)] (M99.3) o *IGL/MYC* [t(8;22)] (M99.4)	X
Burkitt Lymphoma (BL)			

	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for: o *MYC* gene rearrangement (M96.1) o *IGH/MYC* [t(8;14)] (M96.2) o *IGK/MYC* [t(2;8)] (M96.3) o *IGL/MYC* [t(8;22)] (M96.4) o *BCL2* gene rearrangement (M96.5) o *BCL6* gene rearrangement (M96.6)	X
Burkitt Like Lymphoma with 11q			
	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for 11q copy number (M97.1)	X
T-Prolymphocytic Leukaemia (T-PLL)			
	BM & PB	-Karyotype -FISH for: o *TCL1* gene rearrangement (M113.1) o *TRA/TRD* gene rearrangement If G-banding fails, additional FISH for: o Isochromosome 8q (M113.2) o *TP53/DEP17*	*TP53* targeted NGS panel (upon request) [8,9]
Anaplastic Large Cell Lymphoma (ALCL)			
	• FFPE sections • Touch preps • Other sample type as required and infiltration confirmed	FISH for: o *ALK* gene rearrangement (M182.2) o *IRF4/DUSP22* gene rearrangement (M112.3) o *TP63* gene rearrangement (M112.4)	X
Hairy Cell Leukaemia (HCL)			
	PB/BM/other	X	*BRAF* V600 hotspot (NGS) [8,9]
BMT patients (sex-mismatched)			

	BM	FISH for *CEPX/CEPY*	X
Hodgkin lymphoma			
	X	X	X
Histiocytosis **Histiocytic sarcoma**			
	X	X	X
T cell Non-Hodgkin Lymphomas			
	X	X	X
Sarcomas and solid tumors			
	• FFPE sections • Touch preps • Other sample type as required	FISH for: o *EWSR1* gene rearrangement o *SS18* (formerly *SYT*) gene rearrangement o *FOXO1* gene rearrangement o *FUS* gene rearrangement o *ETV6* gene rearrangement o *USP6* gene rearrangement o *MDM2* copy number o *TFE3* gene rearrangement o *NTRK1/NTRK2/NTRK3* gene rearrangement Additional probes can be tested upon request	X

1. MPN-panel which uses targeted amplicon NGS to analyse the hotspots on the three candidate MPN genes *JAK2*, *CALR* and *MPL*. The assay provides information on all 3 genes simultaneously rather than requiring sequential testing
2. The test is performed using the Illumina TruSight Myeloid Sequencing Panel platform; the panel includes 54 genes and further details can be found at at: http://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet-trusight-myeloid.pdf. Genes included in the panel: *ASXL1, ATRX,* ***BCOR, BCORL1****, BRAF, CALR, CBL, CBLB, CSF3R,* ***CUX1, DNMT3A, ETV6, EZH2****, FBXW7, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2,* ***IKZF1****, JAK2, JAK3, KIT, KMT2A, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA,* ***PHF6****, PTEN, PTPN11,* ***RAD21, RUNX1****, SETBP1, SF3B1, SMC1A, SMC3, SRSF2,* ***STAG2****, TET2, TP53, U2AF1, WT1,* ***ZRSR2****.* **Genes in bold, whole gene covered.**
3. https://dna-diagnostic.com/files/Downloads/HemaVision/Manual-HV03-9;22_rev2017.03.pdf
4. https://dna-diagnostic.com/products/hema-vision/screening-for-28-translocations/
5. Performed by Prof. Nick Cross' Leukaemia Research group
6. Performed on any samples referred for eosinophilia by Prof. Nick Cross' Leukaemia Research group at no extra cost; however, results from this test will only be reported if showing a positive result.
7. ***FLT3*** **and** ***NPM1*** **fragment analysis**

The principle of this assay is the discrimination of the wild type from the mutated DNA sequences based of the length of a PCR fragment generated by amplifying regions of the two genes which include the hotspot for insertion/duplication. Mutated genes give rise to larger PCR amplicons and wild type genes produce an amplicon of a predictable and stable size. Two primers are used for each gene. The forward primer in each case is labelled with a fluorescent dye which is incorporated into the amplified PCR product and can later be detected on a capillary electrophoresis instrument during the sizing portion of the assay. Although the PCR reactions and method used to detect amplicons are not fully quantitative, this assay is at least semi quantitative in giving a good estimation of the quantity of mutant alleles present in the starting sample for FLT3-ITD.

8. **Targeted NGS panel for *FLT3*-TKD, *NPM1*, *IDH1*, *IDH2*, *TP53, BRAF* V600, *KIT*.** Targeted amplicon NSG panels designed to sequence gene sub-regions (hot spots) *FLT3*-TKD, *NPM1*, *IDH1*, *IDH2*, *TP53, BRAF* V600. The assay limit of detection is 1%.
9. NGS assays on DNA extracted from formalin-fixed paraffin embedded (FFPE) material are out of scope with respect to UKAS accreditation requirements.