Oncology - Tests Performed According to Disease Entity

The Wessex Genomics Laboratory Service (WGLS) is a combined service across two sites at Salisbury and Southampton (formerly Wessex Regional Genetics Laboratory and Molecular Pathology, respectively). The laboratories provide tests for haematological malignancies, solid organ cancers and pharmacogenomics, aligned with the National Genomics Test Directory.

The oncology section provides genomic analysis of samples from patients with a range of haematological malignancies, lymphoproliferative conditions and solid tumours. 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 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 test indicated in the table below are undertaken according to the National Genomics Test Directory (NGTD) for Cancer (https://www.england.nhs.uk/publication/national-genomic-test-directories/) and, unless otherwise specified, agreed diagnostic algorithms.

NHS England (NHSE) specifies target turnaround times (TATs) for genomic testing for cancer samples through the National Genomic Test Directory and the Genomic Medicine Service to ensure timely delivery of results that can inform patient management. The target TAT varies according to the clinical urgency, test methodology and indication, ranging from **3 days for ultra-rapid urgent tests** to **42 days for complex testing such as whole-genome sequencing**. For most routine cancer genomic investigations, including NGS panel testing for haematological and solid tumours, the target TAT is **21 calendar days**, while selected urgent molecular and cytogenetic tests (e.g. acute leukaemia FISH) have targets of **3 days** to support time-critical treatment decisions. These nationally defined standards are monitored across the Genomic Laboratory Hubs (GLHs) to promote equitable access to high-quality genomic testing and timely reporting throughout England.

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. For additional information on the WGLS policy on the retention and storage of samples, please contact the laboratory.

Where testing is indicated in both the Southampton and Salisbury sites, separate samples sent direct, where possible, are beneficial.

For any additional information or specific testing request, email shc-tr.WRGLdutyscientist@nhs.net (Salisbury) or wgls_cancergenomics@uhs.nhs.uk (Southampton). For Whole Genome Sequencing (WGS) queries, please email wgs_cancerwgls@uhs.nhs.uk .

Suspected diagnosis	Sample type	WGLS Salisbury (NGTD code when applicable)	WGLS Southampton (NGTD code when applicable)
Leucocytosis/Raised WBC/ Neutrophilia			
	PB/BM	• *BCR::ABL1* FISH (M85.24) • Karyotype [BM; PB on request or if *BCR/ABL1* positive by FISH] (M85.3) • MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Myeloid NGS panel (M85.2) [2,#]	
Eosinophilia			
	PB/BM	• *FIP1L1::PDGFRA* FISH (M85.7) • Karyotype [BM] (M85.3) • Myeloid NGS panel (M85.2) [2,#]	• *TCR* gene rearrangements for lymphocytic hypereosinophilia (M111.2)
Erythrocytosis/Polycythaemia/Raised haematocrit/Thrombocytosis/Raised platelets			
	PB/BM	• FISH for *BCR::ABL1* if there is suspicion of CML (i.e. basophilia & thrombocytosis) (M85.24) • Karyotype [BM 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			
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/BM	• MPN panel (*JAK2*, *MPL* & *CALR*) (M85.1) [1] • Karyotype (M85.3) • Myeloid NGS panel on request (M85.2) [2,#] <u>Additional tests available on request</u>: • FISH for *FIP1L1::PDGFRA* if eosinophilia present (M85.7) • FISH for *BCR::ABL1* if there is suspicion of/to exclude CML (e.g. basophilia with thrombocytosis) (M85.24)	• *JAK2* V617F hotspot MRD (M85.14)
Aplastic anaemia (AA)/MDS			
	PB/BM	• Karyotype [BM] (M82.2)	• *TCR* gene rearrangements (M111.2)

		• FISH [BM if karyotype fails or limited analysis; PB on request]. Minimal FISH panel: -5/5q-, -7/7q- & -17/17p-, *MECOM*/3q b/a (M82.) • SNP array can be undertaken in cases with failed G-banding upon discussion with the laboratory (M82.2) [#] • Myeloid NGS panel (M82.1) [2,#]	
MPN/MDS overlap syndromes			
	PB/BM	• Karyotype [BM] (M224.2) • FISH [if karyotype fails or limited analysis]. Minimal FISH panel: -5/5q-, -7/7q- & -17/17p-, *MECOM*/3q b/a (M224.2) • Myeloid NGS panel (M224.1) [2,#]	• *JAK2* V617F hotspot MRD (M85.14)
Chronic Myeloid Leukaemia (CML) including CP and BC			
Diagnosis. Urgent *BCR/ABL1* FISH, karyotype and RT-qPCR to determine transcript breakpoints. **Post-treatment** *BCR::ABL1* monitoring by RT-qPCR on PB **Progression/transformation/rising level of *BCR::ABL1* transcripts**. Karyotype and FISH. **Testing for *BCR::ABL1* TKD mutations** for patients who either fail to respond to treatment or lose their response. This test performed at the West Midlands Regional Genetics Laboratory in Birmingham. Samples for this test should be sent to the Wessex Genomics Laboratory Service at Southampton who will then forward relevant material to the Birmingham Laboratory.	PB/BM	• FISH for *BCR::ABL1* (M84.3) • Karyotype to confirm t(9;22) and check for additional abnormalities (M84.4) • Additional FISH to clarify/exclude additional abnormalities (when required) (M84.)	• *BCR::ABL1/ABL1* RT-qPCR for *BCR::ABL1* breakpoint characterisation (M84.2) (FISH positive cases at diagnosis only) • *BCR::ABL1/ABL1* RT-qPCR monitoring (M84.2)
Mast cell disorders/ mastocytosis			
	PB/BM (preferable)	• Karyotype [BM, 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)[4] • Myeloid NGS panel for ?SM-AHN (M85.2) [2,#]	
Acute Myeloid Leukemia (AML)			

Diagnosis: All samples referred for AML or a high degree of suspicion of AML are activated for rapid *FLT3*-ITD, *FLT3*-TKD (hotspots) and *NPM1* fragment analysis, G-band chromosome analysis and NGS panels of relevant genes.
Reflex FISH or further molecular testing may also be instigated to further characterise initial findings.
 	
Follow up: appropriate testing will be undertaken depending on diagnostic findings.
 	
Measurable residual disease (MRD) analysis by RT-qPCR (when suitable molecular markers are available) is not performed at this laboratory apart from cases with a *BCR::ABL1* fusion. Samples for MRD testing will need to be sent to the Wessex Genomics Laboratory Service Southampton, where cDNA will be made and forwarded to the appropriate testing centre. Please note that upon detection of a molecular marker, material from the baseline sample will be forwarded automatically to the appropriate testing centre for baseline quantification (unless limited sample is available).
 	
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). WGS is available for all paediatric and TYA (under 25 years of age). For adult patients (>25 years of age), WGS is available where results have expected clinical utility and impact. This includes exhausted standard of care from either a clinical or diagnostic perspective.
	PB/BM	• Karyotype (M80.3)
		• FISH for a number of markers including, but not limited to:
		  ○ *RUNX1::RUNX1T1* [t(8;21)] (M80.30)
		  ○ *CBFB::MYH11* [inv(16)(p13.1q22) (M80.31)
		  ○ *PML::RARA* [t(15;17)] for ?APL (M80.29)
		  ○ *KMT2A* gene rearrangement (M80.32)
		• *FLT3*-ITD fragment analysis (M80.18)
		• *NPM1* exon 11 fragment analysis (M80.22)
		• *FLT3*-TKD hotspot fragment analysis (M80.21) [3,#]
		• *FLT3*-TKD, *IDH1, IDH2, NPM1* & *TP53* targeted NGS panel (M80.23) [4]
		• Myeloid NGS panel (M80.2) [2,#]
			• *BCR::ABL1/ABL1* RT-qPCR for *BCR::ABL1* breakpoint characterisation (M80.13) (FISH positive cases at diagnosis only)
			• *BCR::ABL1/ABL1* RT-qPCR monitoring (M80.13)

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	PB/BM	Analysis of PB depending on percentage of blasts and whether separate BM available or not; if no BM	• *BCR::ABL1/ABL1* RT-qPCR for *BCR::ABL1* breakpoint

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 **SNP array** is now performed on all cases of Acute Lymphoblastic Leukaemia in addition to G-banding analysis. The technical process of this test is undertaken at the West Midlands Regional Genetics Laboratory in Birmingham. For pediatric patients, DNA for this test is extracted from the diagnostic sample which is directly sent to the Birmingham laboratory for Minimal Residual Disease (MRD) monitoring by the treating clinician. For adult patients, DNA is extracted from the sample sent for standard cytogenetic investigations at the Salisbury laboratory and forwarded to the Birmingham Laboratory. **MRD monitoring** using Rearrangement of T-Cell Receptor and Immunoglobulin H Genes, not performed at this laboratory. A separate sample should be sent to the recommended testing centre at the time of diagnosis. **Whole Genome Sequencing (WGS)**: is available for newly presenting AML patients on completion of appropriate documentation and submission of a germline sample (M91.1). WGS is available for all paediatric and TYA (under 25 years of age). Older patients enrolled in the ALLTogether trial are also eligible. For adult patients (>25 years of age), WGS is available where results have expected clinical utility and impact. This includes exhausted standard of care from either a clinical or diagnostic perspective.		received then PB will be processed for cytogenetics/FISH analysis • Rapid FISH for *BCR::ABL1*, *KMT2A* (*MLL*), *ETV6::RUNX1* rearrangements (M91.10; M91.57; M91.36) • Karyotype (M91.2) • Additional FISH panel as required (M91.) • SNP array (M91.2) #	characterisation (M91.9) (FISH positive cases at diagnosis only) • *BCR::ABL1/ABL1* RT-qPCR monitoring (M91.9) • *TCR* and *IG* clonality may be undertaken – please discuss with the laboratory for eligibility before sending
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.	PB/BM/other	• FISH for: ○ *TP53* deletion (M94.4) ○ *ATM* deletion (M94.8) ○ Trisomy 12 (M94.10) ○ 13q14 deletion (M94.9)	• *IGHV* somatic hypermutation (M94.6) • *IG* clonality (M94.5)

IGHV hypermutation studies performed once prior to first line treatment. Subset #2 and subset #8 also reported. **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 loosing response/relapsing/progressing on BTK or BCL2 inhibitors**: Re-assessment of *TP53* status and assessment of resistant mutations in *BTK*, *PLCG2* and *BCL2*.		If Richter's suspected: ○ *MYC* gene rearrangement • *TP53* targeted NGS panel (M94.4) [4] • Lymphoid NGS panel for resistant mutations (M94.1) [2,#]	
Multiple Myeloma (MM)/ Plasma Cell Leukaemia (PCL)/AL Amyloidosis/MGUS			
	BM/ PB (PCL only)/ FFPE (Plasmacytoma)	• FISH: on CD138 separated cells for: ○ *IGH* gene rearrangement (M92.8) ○ *IGH::FGFR3* [t(4;14)] (M92.2) ○ *IGH::CCND1* [t(11;14)] (M92.4) ○ *IGH::MAF* [t(14;16)] (M92.5) ○ *IGH::MAFB* [t(14;20)] (M92.6) ○ *TP53/CEP17* (M92.12) ○ *CDKN2C* (1p32.3)/*CKS1B* (1q21) (M92.10 & M92.11) Upon request: ○ *MYC* gene rearrangement (M92.14) Upon request: • *TP53* targeted NGS panel on DNA extracted from CD138 separated cells (m92.1)[4]	
Suspected Lymphoma			
	PB/BM/other with confirmed infiltration	• Karyotype (M80.3)	• *TCR* and *IG* clonality may be undertaken (M225.3, M225.1) – please discuss with the laboratory for eligibility before sending
MALT lymphoma			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *MALT* gene rearrangement (M107.4) ○ *BIRC3::MALT1* [t(11;18)] (M107.1) ○ *IGH::MALT1* [t(14;18)] (M107.3)	• *IG* clonality (M95.1)

Mantle Cell Lymphoma (MCL)			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *IGH::CCND1* [t(11;14)] (M102.1) ○ *CCND2* gene rearrangement (M102.3) ○ *IGH::CCND3* [t(6;14)] ○ *TP53::CEP17* • *TP53* targeted NGS panel (M102.5) [4,5]	• *IG* clonality (M95.1)
Follicular Lymphoma (FL)			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *BCL6* gene rearrangement (M103.2) ○ *BCL2* gene rearrangement (M103.3) • Lymphoid NGS panel (*CARD11, CREBBP, EZH2, ARID1A, EP300, MEF2B, FOXO1*) (M103.5) [2,#]	• *IG* clonality (M95.1)
High grade B cell lymphoma/DLBCL			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *MYC* gene rearrangement (M99.1) ○ *IGH::MYC* [t(8;14)] (M99.2) <u>If *MYC* rearranged:</u> ○ *BCL2* gene rearrangement (M99.5) ○ *BCL6* gene rearrangement (M99.7) <u>If *MYC* rearranged but not with *IGH*:</u> ○ *IGK::MYC* [t(2;8)] (M99.3) ○ *IGL::MYC* [t(8;22)] (M99.4)	• *IG* clonality (M95.1) • *IGHV* somatic hypermutation if ?Richters (M94.6)
Burkitt Lymphoma (BL)			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *MYC* gene rearrangement (M96.1) ○ *IGH::MYC* [t(8;14)] (M96.2) ○ *IGK::MYC* [t(2;8)] (M96.3) ○ *IGL::MYC* [t(8;22)] (M96.4) ○ *BCL2* gene rearrangement (M96.5) ○ *BCL6* gene rearrangement (M96.6)	• *IG* clonality (M95.1)
High-grade B-cell lymphoma with 11q aberrations			

	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for 11q copy number (M97.1)	
Large B Cell like lymphoma with IRF4 rearrangements			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for *IRF4* gene rearrangement (M98.1)	
Hairy Cell Leukaemia (HCL)			
	PB/BM/other with confirmed infiltration		• *BRAF* V600 hotspot (M108.2) • *IGHV* somatic hypermutation (M108.6) • *IG* clonality (M95.1)
Splenic B-cell lymphoma/leukemia with prominent nucleoli (also includes HCL-variant) & Paediatric type follicular lymphoma			
	PB/BM/other with confirmed infiltration	• Lymphoid NGS panel (*MAP2K1*) (M181.1 & M110.1) [2,#]	•
IgM MGUS/Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinaemia			
	PB/BM/other with confirmed infiltration	• Lymphoid NGS panel (*MYD88, CXCR4*) (M104.1)[2,#]	• *MYD88* hotspot by digital PCR (M104.2/M105.2)
Intra-Ocular Lymphoma			
	Infiltrated material		• *MYD88* hotspot by digital PCR (M106.2)
T-Prolymphocytic Leukaemia (T-PLL)			

	PB/BM/other with confirmed infiltration	• Karyotype (M113.6)
		• FISH for:
		  ○ *TCL1* gene rearrangement (M113.1)
		  ○ Isochromosome 8q (M113.2)
			• *TCR* gene rearrangement (M111.2)

Mycosis Fungoides/Sezary Syndrome			

Comparative clonality studies across specimens	PB/BM/other with confirmed infiltration		• *TCR* gene rearrangement (M111.2)
Anaplastic Large Cell Lymphoma (ALCL)			
	PB/BM/FFPE sections/other with confirmed infiltration	• FISH for: ○ *ALK* gene rearrangement (M182.2) ○ *IRF4/DUSP22* gene rearrangement (M112.3) ○ *TP63* gene rearrangement (M112.4)	
T cell Non-Hodgkin Lymphomas			
	PB/BM/other	• Lymphoid NGS panel (*RHOA, DNMT3A, IDH2, TET2, PLCG1, CARD11, IRF4, POT1, VAV1, PLCG1, FYN*) (M111.1) [2,#]	• *TCR* gene rearrangement (M111.2/M225.2)
Large Granular Lymphocyte Leukaemia, NK Cell/Gamma-Delta T Cell Lymphoma, Hepatosplenic T Cell Lymphoma			
	PB/BM/other	• Lymphoid NGS panel (*STAT3*, *STAT5B*) (M114.1/M115.1/M116.1) [2,#] <u>For Hepatosplenic T Cell Lymphoma only:</u> • FISH for isochromosome 7q (M116.2)	
Hodgkin lymphoma			
	PB/BM/FFPE sections		• *IG* clonality (M95.1)
Histiocytosis / Histiocytic sarcoma			
	PB/BM/FFPE sections		• High sensitivity *BRAF* (M117.19)
BMT patients (sex-mismatched)			
	BM	• FISH for *CEPX/CEPY* (M118.2)	• STR analysis for post allogeneic transplant chimerism monitoring (M118.1)
Sarcomas and solid tumors			
	FFPE sections/ Touch preps/	• FISH for:	• Colorectal cancer:

	Other sample type as required	○ *EWSR1* gene rearrangement (M45.1; M48.1; M66.1) ○ *SS18* gene rearrangement (M77.1) ○ *FOXO1* gene rearrangement (M42.1) ○ *FUS* gene rearrangement (M45.1; M64.1; M76.1) ○ *ETV6* gene rearrangement (M17.1; M62.1) ○ *USP6* gene rearrangement (M69.1) ○ *MDM2* copy number (M79.1) ○ *TFE3* gene rearrangement (M18.1) ○ *NTRK1/NTRK2/NTRK3* gene rearrangement (M244.) <u>Additional probes can be tested upon request</u>	○ Multi target NGS panel essential targets (M1.1) ○ MSI testing (M1.4) ○ *MLH1* promoter hypermethylation (M1.5) ○ *DPYD* actionable variants (M1.7) • Breast cancer ○ *PIK3CA* (M3.6) [6] ○ *DPYD* actionable variants (M3.7) • Non-Small Cell Lung cancer ○ Multi target NGS panel essential targets (M4.1) • Melanoma ○ Adult melanoma - Multi target NGS panel essential targets (M7.1) ○ Uveal melanoma - Multi target NGS panel (M187.2) • Papillary thyroid carcinoma ○ *BRAF, KRAS, NRAS* (M9.1) [6] • Cancer of unknown primary ○ *DPYD* actionable variants (M226.3)

			• Solid tumour other (i.e. specific histology not listed elsewhere in the test directory) ○ *DPYD* actionable variants (M227.3) <u>This is not an exhaustive list of all DPYD indications</u>

1. MPN-panel which uses targeted amplicon NGS to analyse the hotspots on the three candidate MPN genes *JAK2*, *CALR* and *MPL*. It is now our policy to simultaneously test for *JAK2* V617F, *CALR*, *MPL* and *JAK2* exon 12 on all samples referred for any of these tests.
2. Library preparation, sequencing and analysis is undertaken at the West Midlands Regional Genetics Laboratory (WMRGL), Birmingham. Analysis and reporting is undertaken at the Wessex Genomics Laboratory Service – Salisbury.
3. For *FLT3*-TKD hot-spots: a combination of fragment analysis by PCR and EcoRV restriction digest is used for the detection of *FLT3* tyrosine kinase domain (TKD) variants at codons Asp835 (nucleotide position c.2503 to c.2505) and Ile836 (nucleotide position c.2506 to c.2508). This assay does not exclude the presence of other pathogenic variants within the TKD and is non-quantitative, therefore; an independent *FLT3* assay using NGS technology is run in parallel to give more comprehensive coverage of the *FLT3*-TKD and provide confirmation and estimation of allelic burden for any variants detected by this assay.
4. Targeted amplicon NSG panels designed to sequence gene sub-regions (hot-spots) of *FLT3*-TKD, *NPM1*, *IDH1*, *IDH2* and *TP53.*
5. NGS assays on DNA extracted from formalin-fixed paraffin embedded (FFPE) material are out of scope with respect to UKAS accreditation requirements.
6. Current panel is not fully compliant with the National Genomics Test Directory test specification. Expansion of the panel is in progress.

This technique is out of scope with respect to UKAS accreditation requirements.

NGS. Next Generation Sequencing; PB, peripheral blood; BM, bone marrow; FFPE, formalin-fixed paraffin-embedded.