Oncology - Tests Performed According to Disease Entity

The table below lists the disease categories that we deal with, what type of tissue should be sent for each and when in the disease course to send it. This latter is only a guide. Our main criterion for accepting a sample is that the result will be clinically useful. If in doubt, either phone to discuss the case or make it clear on the referral form precisely why you are requesting the test.

Condition confirmed/suspected	Material	Karyotype	FISH	Molecular testing
CML at diagnosis	BM or PB	**Yes**	**Only in specific circumstances:** *BCR/ABL1* FISH if: • Sample received is PB • G-banding failed • Variant translocation • Specifically requested • G-banding is known to be delayed	• Targeted RT-PCR[1] to confirm breakpoints
CML routine follow-up[2]/ disease acceleration/ rising level of *BCR-ABL1* transcript	-BM -PB only for FISH if BM not available	**Yes**	**Only in specific circumstances:** *BCR/ABL1* FISH if: • G-banding failed • Only PB available • Specifically requested	
AML diagnosis	-BM (Please remember KCH tube as well) -PB if aspirate is dry tap & if it contains any leukaemic cells	**Yes**	**Only in specific circumstances:** • Rapid FISH for *PML/RARA* [t(15;17)] if ?APL • On request: Del(5q), del(7q), *TP53* status if G-banding failed or limited analysis	• RT-PCR[1] (if eligible for BMT[3]) • For core binding factor AML: *KIT* testing with extended NGS panel (exons 2, 8, 9, 10, 11 & 17) • AML panel: *FLT3*-ITD, *FLT3*-TKD, *NPM1, IDH1/2* • Myeloid NGS panel [8]
AML follow up	BM	**Yes, if previous sample abnormal.** Once normal karyotype restored, samples are only accepted if there	**Only in specific circumstances:** • FISH for previously identified abnormalities (if probe available) if G-banding failed	• For core binding factor AML: *KIT* testing with extended NGS panel (exons 2, 8, 9, 10, 11 & 17) • *FLT3*-ITD, *FLT3*-TKD, *IDH1*, *IDH2* • Myeloid NGS panel[8]

		is clinical concern of ?relapse		
ALL (B and T) diagnosis	-BM (Please remember KCH as well) -PB if aspirate is a dry tap, if it contains any leukaemic cells	**Yes**	• *ETV6/RUNX1* in paediatric B ALL to exclude iAMP21 • *BCR/ABL1* in adult ALL • Extensive FISH panel to look for ploidy changes or specific rearrangements if G-banding fails or equivocal (for specific rearrangements, contact the laboratory) • *ABL1* to exclude *ABL1* amp in T-ALL	• RT-PCR[1] (if eligible for BMT[3])
ALL follow up	BM	**No** Unless ?relapse	**Yes** if marker detectable with specific FISH probe present at diagnosis	**No**
MDS & MDS-MPN[5]	-BM for cyto -PB for molecular testing	**Yes**	**Only in specific circumstances:** • del(5q) and del(7q) on failed G-banding (on request).	• Myeloid NGS panel[8]
MPN	-BM for cyto and **preferred** for *KIT* -PB for all other molecular testing	**Yes** (when requested)	**Yes** *BCR-ABL1* (when requested)	• *JAK2* p.(Val617Phe) and *JAK2* exon 12, *MPL* and *CALR*[5] • Myeloid NGS panel[8]
Eosinophilia	BM - FISH/cyto/molecular testing PB - FISH/cyto/molecular testing DNA only molecular testing	**Yes** Only if *FIP1L1-PDGFRA* negative on BM sample	**Yes** *FIP1L1-PDGFRA*	• *FIP1L1-PDGFRA*[10] • *STAT5B* and *JAK2* exon 13[11] • Myeloid NGS panel[8]
Eosinophilia (monitoring)	BM or PB or cDNA	**No**	**No**	• RT-PCR for known rearrangements like *FIP1L1-PDGFRA* and other (e.g., *ETV6-PDGFRB*, *SPEC-FLT3*, *BCR-PDGFRA*, etc.)[9]
Mastocytosis	BM (preferably) PB	**No**	**No** Exception: if associated haematological disorder suspected, G-banding can be performed	• *KIT* D816V by digital PCR • If *KIT* D816V negative or young patient, extended NGS panel (exons 2, 8, 9, 10, 11 and 17) • For patient <18 years: additional

				automatic testing for *KIT* K509I and *KIT* D419del[9] • Myeloid NGS panel[8]
Hairy Cell Leukaemia	PB	**No**	**No**	• *BRAF* p.(Val600Glu)
Hodgkins	-	**No** Unless there is a question of secondary MDS/AML	**No**	**No**
ITP	-	**No**	**No**	**No**
Aplastic anaemia	BM	**Yes**	**No** Exception: del(5q) and del(7q) on failed G-banding (on request)	• Myeloid NGS panel[8]
BMT patients (sex-mismatched)	BM	**Yes**	**Yes** *CEPX/CEPY*	**No**
LPD	-PB -BM is accepted but PB is preferred	**No** Exception: Richter's transformation (on request)	**Yes** *IGH/CCND1* [t(11;14)] *ATM*, *CEP12*, *13q*, *TP53*	• *TP53* using amplicon-based NGS
Lymphoma (staging BM)		**No**	**No** Exception: lymphocytosis confirmed (on request) see below for specific probes	**No**
Lymphoma	- Biopsy impression smears -Biopsy paraffin embedded tissue sections	**No** **Exceptional circumstances:** 1) Fresh lymph nodes can be cultured and analysed by G-banding upon special request 2) If sample type is confirmed infiltrated BM and the referral reason is ? Burkitt	**Yes** **Probes available (for additional probes not listed below contact the laboratory):** • *IGH-CCND1* [t(11;14)] • *CCND2* breakapart • *IGH-CCND3* [t(6;14)] • *BCL2* break apart • *BCL6* break apart • *MYC* break apart • *IGH-MYC* [t(8;14)]	• *TP53* using amplicon-based NGS **for MCL**

			• *IGK/IGL/MYC* dual fusion • *MALT* break apart • *BIRC3-MALT1* [t(11;18)] • *IGH-MALT1* [t(14;18)] • *ALK* break apart	
Multiple Myeloma	BM	**No**	**Yes** (on CD138+ separated cells) **Probes available:** • *IGH* break apart • *IGH-FGFR3* [t(4;14)] • *IGH-CCND1* [t(11;14)] • *IGH-MAF* [t(14;16)] • *IGH-MAFB* [t(14;20)] • *TP53/CEP17* • *CDKN2C* (1p32.3)/*CKS1B* (1q21) **Upon special request:** • *MYC* breakapart	**No**
Sarcoma[7]	-Fresh tissue -Paraffin embedded tissue sections -Impression smears	**Yes**	**Yes** **Probes available[6]:** • *EWSR1* break apart • *SS18* (formerly *SYT*) break apart • *FOXO1* break apart • *FUS* break apart • *ETV6* breakapart	**No**
Paediatric solid tumours[7]	-Fresh tissue -Impression smears	**Yes**	**Yes[6]**	**No**
Bladder Cancer	Fresh Urine	**No**	**Yes (UroVysion)**	**No**

NGS. Next Generation Sequencing

[1]RT-PCR: HemaVision Screen Kit able to detect 28 different gene rearrangements commonly seen in leukaemia (http://dna-diagnostic.com/products/hemavision/screening-for-28-translocations/).

[2]Chromosome analysis is used to monitor the initial response to treatment until complete cytogenetic remission (CCyR). However, more sensitive molecular methods are required to monitor patients after cytogenetic remission is achieved (please note that quantitative RT-PCR testing is carried out at the Molecular Pathology Laboratory at Southampton General Hospital, not at WRGL).

[3] Patient's age ≤ 70 years.

[4] We will not refuse repeat samples on untreated patients provided there has been a reasonable interval since the previous sample (at least 6 months).

[5] Our policy is to simultaneously test for *JAK2* V617F, *JAK2* exon 12, *CALR* and *MPL* on all MPN samples referred for any of these tests.

[6] FISH for some of the rare tumours can be undertaken with prior agreement with the laboratory.

[7] Fresh tumour biopsy with accompanying touch preps if possible. Where material is limited and the likely diagnosis has a specific chromosomal rearrangement, touch preps alone are acceptable. Diagnostic BM samples are also accepted where the tumour sample has not been sent. These must be followed up by phone or fax to say whether or not the marrow is involved.

[8] 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. Main referral reasons: (i) Patients with persistent unexplained cytopenia; (ii) patients with persistent unexplained eosinophilia; (iii) patients with myelofibrosis or systemic mastocytosis to identify those who are likely to have a poor prognosis; (iv) cases with triple negative MPN; (v) MPN suspected of transforming to myelofibrosis or acute leukaemia to provide evidence of progression/transformation.

[9]Performed by Prof. Nick Cross' Leukaemia Research group

[10] FISH for *FIP1L1-PDGFRA* can miss a small proportion of *FIP1L1-PDGFRA* positive cases, as reported by Olsson-Arvidsson *et al*. in the British Journal Haematology 2019 (doi: 10.1111/bjh.16340). In view of these observations, multiplex genomic DNA analysis (able to detect >95% of positive cases) will also be 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 discordant with FISH.

[11] 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.