University Hospitals Bristol NHS
NHS Foundation Trust

Molecular Haematology Oncology Diagnostics

A User's Guide

UHBristol
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CONTENTS

Laboratory Contact Information

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Telephone

0117 342 2596 Specimen notification and general queries
0117 342 2596 Flow Cytometry queries & results
0117 342 0779 SiHMDS office/Molecular Laboratory queries & results

Postal and Visiting Address

SiHMDS
Department of Haematology
Level 8, Queen's Building
Bristol Royal Infirmary
Upper Maudlin Street
Bristol BS2 8HW

Accreditation and Quality Assurance

The Pathology Laboratories are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 15189 standards.
We participate in the UK NEQAS scheme and subscribe to all their available schemes: Factor V Leiden, Prothrombin gene variant, HFE gene analysis, JAK2, Leukaemia-associated chromosome abnormalities and BCR-ABL quantification, BRAF, KIT D816V, NPM1 and FLT3 additionally we participate in the NEQAS BCRABL1 kinase domain variant programme which is a pilot. The Molecular laboratory participates in sample exchange schemes for IgV_H and MYD88 to ensure quality.

Specimen Requirements and Transport

Specimen requirements may vary depending on the patient's WBC count, absolute lymphocyte count, the number of immature or abnormal cells present, the patient's clinical history, the type of tissue being sampled, etc. For these reasons, we ask that all tests be scheduled with the laboratory by calling 0117 342 2596. We will consult with the caller to determine exact specimen requirements, the most appropriate method of handling and transport.

General guidelines:

For non-urgent samples, specimens need to arrive on a Monday -Thursday, no older than 48 hours. Samples that arrive later than 15.00 are normally processed the following day. Therefore it is best to not send specimens on a Friday.
EDTA (purple top) anti-coagulated peripheral blood and bone marrow is acceptable for all flow and molecular analysis.
Specimens are triaged according to clinical urgency and the diagnostic assays are selected based on provisional diagnosis.
Urgent results are usually telephoned and so the relevant contact information is important.

Address:

SiHMDS Reception
Department of Haematology
Level 8, Queen's Building
Bristol Royal Infirmary
Upper Maudlin Street
Bristol BS2 8HW

Please state "**URGENT SPECIMEN FOR FLOW AND MOLECULAR ANALYSIS**" on the parcel.

Urgent Cases

These are cases where a result is urgently needed, such as acute leukaemia. Some cases require RNA preparation, such as BCR-ABL, where the material deteriorates rapidly and in this case the samples also require urgent analysis.

It is imperative that the SiHMDS reception (flow lab) is alerted to the transport of urgent samples. The lab should be telephoned on 0117 342 2596; internally use should be made of the Careflow system.

Any specimens with an urgent need for results will be reported by telephone, or, if required, by secure e-mail, within two hours of specimen receipt.

Reports

Urgent molecular queries are reported within one working day of sample receipt where possible. All leukaemia and lymphoma findings are discussed with the clinical lead in a weekly laboratory MDT.

Routine Molecular reports are generally issued within 14 days of specimen arrival. Please contact the laboratory for more urgent processing in exceptional circumstances.

The integrated reports for bone marrows are normally issued within 14 days of specimen receipt. These include the individual evaluations of morphology, flow cytometry, molecular haematology, trephine biopsy together with cytogenetics, where available.

Reports can be sent out by royal mail or electronically, as preferred, or viewed on ICE.

Molecular Haematology

Tests

Factor V Leiden

Factor V Leiden (Arg506Gln) is a prothrombotic disorder which increases the susceptibility to venous thrombosis by inducing activated protein C resistance. It is due to a G to A transition of nucleotide 1691 in exon 10 of the factor V gene. It has been identified in 3.5% of the UK population and, in heterozygous form, increases the risk of thrombosis 8 fold. Homozygous factor V Leiden is found in 1 in 1600 individuals and increased venous thrombotic risk by 80 – 100 fold. The defect can be identified in approximately 20% of individuals with de novo venous thrombosis and accounts for 40% of familial thromboembolism.

Factor V Leiden analysis is commonly performed as part of a thrombophilia screen. Samples are screened for activated protein C resistance using a phenotypic assay and positive patients are confirmed by genotype. The criteria for selecting patients for such testing and information sheets for patients carrying the defect are available from the Haematology Department – Please contact the Clinical Haematology Coagulation team.

Analysis method

Melting curve analysis using fluorescent probes and LightCycler technology.

Results reported

- Factor V Leiden = Normal
- Factor V Leiden = Homozygous
- Factor V Leiden = Heterozygous

Prothrombin gene variant G20210A

This molecular defect occurs in the 3' untranslated region of the prothrombin gene and is associated with a 30% increase in plasma prothrombin levels. The variant is found in 1.5% of Northern European individuals and the heterozygous state is associated with a 3 fold increased risk of venous thrombosis. Prothrombin gene variant analysis is generally performed as part of a thrombophilia screen. The criteria for selecting patients for such testing and information sheets for patients carrying the defect are available from the Haematology Department– Please contact the Clinical Haematology Coagulation team.

Analysis method

Melting curve analysis using fluorescent probes and LightCycler technology.

Results reported

- G/A = Heterozygous

Results suggest a weak risk factor for thrombosis. If required, further advice is available from the Department of Haematology.

Hereditary Haemochromatosis

Hereditary haemochromatosis is a common autosomal recessive genetic disorder which is associated with iron overload. Clinical manifestations include cardiomyopathy, liver disease progressing to cirrhosis, arthropathy and diabetes mellitus. 90% of patients are homozygous for a missense variant in the HFE gene (C282Y) and 5% of cases are compound heterozygous for C282Y and a second variant, H63D. 1 – 2% of cases of hereditary haemochromatosis are due to a rare variant, S65C, which may be present as a heterozygous defect alone or compound heterozygous with C282Y or H63D.

The carrier rate for the C282Y allele in the UK population is 9%. Population studies have shown that the penetrance of overt clinical disease in C282Y homozygotes is relatively low.

HFE gene variant analysis is commonly performed to screen for hereditary haemochromatosis and for performing family studies. The test should be combined with phenotypic assays for serum ferritin and transferrin saturation (clotted blood sample) to detect biochemical evidence of iron overload.

Analysis Method

Melting curve analysis using fluorescent probes and LightCycler technology.

Results reported

HFE gene analysis for C282Y, H63D and S65C variants:

1. Normal wild type
   (No variants detected)
2. C282Y heterozygous
   The carrier frequency for the C282Y variant in the local population is 9%.
3. C282Y homozygous
   The carrier frequency for the C282Y variant in the local population is 9%.
4. C282Y homozygous
   This genotype is identified in 90% of patients with hereditary haemochromatosis.
5. C282Y/H63D compound heterozygous
   This genotype is found in approximately 5% of patients with hereditary haemochromatosis.
6. S65C heterozygous
   Heterozygous S65C (alone or in combination with C282Y or H63D) may result in mild to moderate iron overload.
7. H63D heterozygous
   This genotype is not associated with a significantly increased risk of iron overload.
8. H63D homozygous
   This genotype is not associated with a significantly increased risk of iron overload.

Thiopurine Methyltransferase Variant Screening

The enzyme thiopurine methyltransferase (TPMT) catalyses the metabolism of the widely used thiopurine-based group of drugs which include 6-mercaptopurine, azathioprine and 6-thioguanine. These agents are used in the treatment of acute leukaemias and as immunosuppressants for the management of organ transplantation and autoimmune disorders. Clinical toxicity of these drug leads to bone marrow suppression and damage to the gut. Several common sequence variations in the TPMT gene have been identified and are associated with reduced enzyme activity and increased drug toxicity. Individuals homozygous or compound heterozygous for these variants (0.3% of the UK population) have severe TPMT deficiency and are at high risk of developing severe bone marrow aplasia following exposure to thiopurine agents. Heterozygous carriers may also be more sensitive to these drugs and require more careful haematological monitoring.

Patients should be screened for TPMT enzyme deficiency (2 x EDTA blood samples sent to Clinical Chemistry Department) prior to commencing the above agents. As the enzyme is assayed in red cells, however, a false negative result may be obtained if the patient has received a blood transfusion in the previous 3 months. The PCR-based test may be useful in this situation and also in urgent clinical cases when a result can be made available within 48 hours. Genotypic analysis for the two commonest mutant alleles G460A and A719G can be performed in this situation and will identify 80 – 90% of patients at risk of severe marrow toxicity. At present this assay is available for all paediatric and TYA ALL patients on trial (as a trial requirement) – in any other situations we recommend testing the enzyme level in the first instance; if there are special reasons for requesting this test (e.g post-transfusion) please contact the lab on 0117 342 0779.

Analysis Method

Restriction endonuclease digestion-PCR and Sanger sequencing

Results reported

- Wild type allele: 1
- Mutant alleles: 2 (G238C), 3A (G460A + A719G), 3B (G460A), 3C (A719G)
    - TPMT genotype 1/1: normal wild type
    - TPMT genotype 1/3C: heterozygous
    - TPMT genotype 3A/3A: homozygous

Heterozygous individuals may show increased sensitivity to azathioprine and require careful haematological monitoring during initiation of therapy. Individuals who are homozygous for the mutant alleles are at risk of severe toxicity at standard dosage regimes. Current methods detect about 80% of TPMT variants in N Europeans.

Jak2 variant screening in myeloproliferative disorders

The diagnosis of myeloproliferative disorders can be difficult and is often by exclusion. However, a specific point variant in exon 14 of the Jak2 gene resulting in a V617F amino acid substitution has recently been identified in 90% of patients with primary polycythaemia vera and 50 – 60% of cases of essential thrombocythaemia and idiopathic myelofibrosis. The defect may also be found in very small numbers of patients with chronic myelomonocytic leukaemia, myelodysplastic syndrome and acute non-lymphoblastic leukaemia. The variant may be heterozygous or homozygous.

Jak2 variant screening is an appropriate test to request in patients with polycythaemia (haematocrit > 0.52 in males and > 0.48 in females) or a thrombocytosis (platelet count > 500 x 10^9/L) in whom secondary or reactive causes of the blood abnormality have been excluded. Secondary polycythaemia is commonly associated with chronic pulmonary disease, renal disease and some solid tumours (eg hepatomas, renal cell carcinoma, and large uterine fibroids). A reactive thrombocytosis may be due to bleeding, inflammatory disorders or malignancy and commonly develops following surgery. It is important to emphasise that a negative test does not exclude a myeloproliferative disorder. A small proportion of patients with polycythaemia vera and patients with myelofibrosis and essential thrombocythaemia have acquired variants in Jak2 exon 12 and the thrombopoietin receptor gene (Mpl), respectively. Approximately 7% of individuals of African descent have a polymorphism in Mpl (Mpl Baltimore) which is associated with a mild familial thrombocytosis and can be mis-diagnosed as myeloproliferative disease. Methodology is available to test for these variants in selected patients. Discussion with the laboratory is advised prior to requesting these tests.

Analysis Method

Melting curve analysis using fluorescent probes and LightCycler technology. This method detects a clonal population of Jak2+ cells down to a sensitivity of 3%. Weak positive results are confirmed with a second allele-specific oligonucleotide (ASO) technique.

Results reported

1. Jak2 variant (Val617Phe) negative
2. Jak2 variant (Val617Phe) positive

Published data indicates that the Jak2 variant is positive (heterozygous or homozygous) in 90% of cases of polycythaemia rubra vera and 50% of patients with idiopathic myelofibrosis and essential thrombocythaemia.

FLT3 variants in Acute Myeloblastic Leukaemia (AML)

FLT3 is a class III receptor tyrosine kinase. Two types of activating variants have been described in this gene in ANLL: an internal tandem duplication (ITD) of the juxtamembrane domain coding sequence and missense variants of D835 within a kinase domain. Such variants have been identified in approximately 25% of cases of ANLL with a particularly high frequency in patients with normal cytogenetics, hyperleukocytosis and monoblastic differentiation. In this group, it has been shown that FLT3 ITD variants are a significant negative prognostic indicator and a major independent predictor of relapse. FLT3/ITD is an independent risk factor for survival, although less important than cytogenetics and age. The prognostic significance of D835 variants is less certain. FLT3 variants are also found at high frequency in acute promyelocytic leukaemia (45%) but do not appear to impact on relapse risk or survival in this variant.

Analysis Method

PCR and agarose gel electrophoresis

Results reported

1. FLT3 internal tandem duplication: positive (ITD size)

FLT3 length variants are found in 25% of adult AML patients. The variant is associated with a higher relapse rate and, in most studies, shorter overall survival.

Nucleophosmin (NPM1) gene variants in AML

Nucleophosmin (NPM1) is a nucleolar phospoprotein with four main functions: promotion of the biogenesis of the ribosome, control of centrosomal duplication, modulation of tumour suppressor transcription factors and regulation of various nuclear proteins. Recently, heterozygous NPM1 variants have been identified in 35% of adult patients and 6.5% of children with acute myeloblastic leukaemia (AML). In adults, variants are present in 50 – 60% of patients with normal cytogenetics. It is commonest in FAB types M4 and M5.

Although the genetic variants are heterogeneous, virtually all the defects occur in exon 12 and cause a frameshift in the region encoding the C terminus of the NPM1 protein. About 40 molecular variants of NPM1 variants have been described to date in AML patients with >95% occurring at nucleotide position 960. The most common variant (Type A) duplicates a TCTG tetranucleotide at positions 956 to 959 of the reference sequence (GenBank accession number NM_002520), and accounts for 75% - 80% of adult NPM1+ AML cases. The second commonest, Type B is found in 12% of cases.

Early clinical studies indicate that a combination of NPM1 and FLT3 variant status provides a strong prognostic marker in AML patients with normal cytogenetics. NPM1 variants without concomitant FLT3-ITD identify a subgroup of AML (normal karyotype) patients with a

favourable prognosis and has been associated with an approximately 60% probability of survival at 5 years in younger patients. In a donor versus no-donor comparison, the good prognostic group of NPM1-mutated/FLT3 ITD–negative patients did not benefit from allogeneic stem cell transplantation, suggesting that such treatment should not be recommended in first complete remission.

NPM1 variant status is a stable molecular marker and early studies indicate that it will have value in minimal residual disease monitoring.

Analysis method

HRM screening followed by PCR and direct nucleotide sequencing.

Result reported

1. NPM1 variant positive (Type)

NPM1 variants are found in 60% of patients with AML and normal cytogenetics.

In the absence of FLT3 variants, most studies indicate that NPM1 + patients in this sub-group have a significantly better survival (approximately 60% at five years).

Qualitative Molecular Analysis for Leukaemia-associated Chromosome Abnormalities

These are specialist investigations which are of value in the diagnosis of chronic and acute leukaemias. Identification of the common chromosome translocations listed, either by cytogenetic or molecular analysis, is particularly important because they have powerful prognostic significance. Qualitative detection of mutant fusion RNA transcripts may be of value for disease monitoring and assessing response to treatment (eg PML-RARα). However, quantitative real-time PCR provides a more sensitive and robust technique for this purpose and is performed as part of the major national trials.

t(9;22)(q34;q11)

This reciprocal translocation creates the mutant fusion gene BCR-ABL on the 22q- chromosome (Philadelphia) which is identified in the large majority (> 95%) of patients with chronic myeloid leukaemia (CML) and a minority of cases of acute lymphoblastic leukaemia (ALL) of B cell lineage (30% of adult cases and 2 – 5% of children). In CML, two possible breakpoints in the BCR gene yield an e13a2 and/or e14a2 junction in the large majority of cases and the final product is a 210 kDa cytoplasmic fusion protein, p210BCR-ABL. An alternative breakpoint in the BCR gene (e1a2) in ALL generates a p190BCR-ABL product.

t(15;17)(q21;q11)

This translocation is the commonest rearrangement associated with AML subtype FAB M3 (acute promyelocytic leukaemia) and creates a fusion gene between PML1 and RARα.

Analysis method

Reverse transcriptase-PCR

Results reported

Eg: Analysis of AML fusion transcripts by RT-PCR
PML-RARalpha [t(15;17)] = positive

Special Specimen Requirements

As these tests are RNA-based, specimens should be received in the laboratory within twenty four hours of sampling and sent Monday – Thursday.

Quantitative PCR (Q PCR) for p210 BCR-ABL and ABL kinase domain variant analysis

Molecular monitoring has become an important tool in the management of patients with chronic myeloid leukaemia. It enables the response to targeted therapy with imatinib to be accurately determined and informs decisions regarding the introduction of second generation tyrosine kinase inhibitors, the timing of stem cell transplantation and the need for post-transplant treatment with donor lymphocyte infusions (DLIs) in the event of molecular relapse.

The quantitative PCR method employed in our laboratory involves reverse transcription of RNA to cDNA and subsequent use of specific fluorescent hybridisation probes and LightCycler technology. Standardised methods are used and have been developed in collaboration with UK and German reference centres.

As a member of the BCR-ABL Harmonisation network, our laboratory will had the early opportunity to trial the Q-PCR international standards which are in development and these are now in routine use for this assay.

Results reported

The following information is reported:

- BCR-ABL transcript type (where available).
- ABL transcripts/ 2µl cDNA
- BCR-ABL transcripts/ 2µl cDNA
- BCR-ABL/ABL ratio (%)

Serial values for individual patients will be provided as hard copy.

Interpretation

Positive Q-PCR results are expressed as the BCR-ABL/ABL ratio: a value of 1.0% is approximately equivalent to achievement of complete cytogenetic remission (CCR) and 0.1% to major molecular remission (MMR). For negative specimens, the number of ABL transcripts gives an indication of the sensitivity with which BCR-ABL can be excluded for that particular specimen. eg a value of 10,000 indicates that BCR-ABL can be excluded with a sensitivity of $1x10^{-4}$.

It is recommended that molecular monitoring is performed at three monthly intervals following the commencement of TKI therapy.

Variant analysis is recommended in case of failure to achieve a complete haematological response after three months of imatinib therapy, a major cytogenetic response (<35% Ph chromosomes) after six months, a complete cytogenetic response after 12 months or a major molecular response after 18 months. Loss of these responses, progression to accelerated phase or blast crisis or a significant rise in BCR-ABL should also initiate variant analysis.

Special Specimen Requirements

As these tests are RNA-based, specimens should be received in the laboratory within twenty four hours of sampling and sent Monday – Thursday. Please provide at least 10ml of peripheral blood.

ABL kinase domain (AKD) variant analysis

AKD variant analysis is performed in our laboratory by nested reverse transcriptase PCR and direct sequencing. This method has a sensitivity of around 20%. The significance of very small subclones carrying kinase domain variants is uncertain.

In line with proposed guidelines from other Networks, we propose to trigger AKD variant analysis:

1. In response to clinical request
2. Automatically if treatment failure is identified at 3 or more months
3. Automatically if treatment response remains sub-optimal at 12 or 18 months (eg failure to achieve CCyR at 12 months or MMR at 18 months).
4. Automatically on loss of response after a confirmed increase of more than 0.5 logs
5. Automatically on confirmed re-emergence of molecular residual disease

Where variants are reported, we would plan to include a profile of known sensitivity or resistance to available tyrosine kinase inhibitors.

Ph+ acute lymphoblastic leukaemia

By simple modification, the Q-PCR molecular monitoring technique for CML employed in our laboratory can be utilised to provide quantitative results for the e1a2 BCR ABL variant found in Ph+ acute lymphoblastic leukaemia. This method has been established in our Unit and preliminary data on molecular monitoring in local patients is accumulating. For further information, please contact laboratory.

Detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspected lymphoproliferative disorders

In most patients with suspected lymphoproliferative disorders, histology, immunocytochemistry and flow cytometry can confirm the diagnosis of malignancy. In 5 – 10% of cases, however, there may be uncertainty regarding the neoplastic nature of the

proliferation and molecular tests to detect monoclonality can be very helpful in reaching a diagnosis.

The majority of lymphoid malignancies belong to the B-cell lineage (90–95%) with only a minority being T-cell (5–7%) or NK-cell lineage (<2%). Acute lymphoblastic leukemias (ALLs) are of T-cell origin in 15–20% of cases, but in the group of mature lymphoid leukemias and in non-Hodgkin lymphomas (NHLs) T-cell malignancies are relatively rare, except for specific subgroups such as cutaneous lymphomas The vast majority of lymphoid malignancies (>98%) contains identically (clonally) rearranged immunoglobulin (Ig) and/or T-cell receptor (TCR) genes, and in 25–30% of cases well-defined chromosome aberrations [eg t(14;18), t(11;14)] are found, all of which can serve as markers for clonality. These markers can be detected by PCR-based methods. In recent years, standardised techniques have been published by the European Biomed-2 Concerted Action Group and their use has been promoted as "best practice" for larger laboratories routinely involved in leukaemia/lymphoma diagnosis. Reagents and oligoprimers for these methods are commercially available.

Molecular clonality diagnostics in hemato-oncology may have the following indications:

- Any suspect B-cell proliferation when morphology and immunophenotyping are not conclusive.
- All suspect T-cell proliferations (caution: T-cell-rich B-NHL).
- Lymphoproliferations in immunodeficient patients, including post-transplant patients.
- Evaluation of the clonal relationship between two lymphoid malignancies in one patient or discrimination between a relapse and a second malignancy; further classification of a malignancy, for example, via Ig/TCR gene rearrangement patterns or particular chromosome aberrations; occasionally, staging of lymphomas.

Analysis method

Commercial InVivoScribe IdentiClone kits (CE marked) are used to perform these assays and involve multiplex PCR and clone detection by agarose gel electrophoresis.

For IgH clonality analysis, complete VH–JH, incomplete DH–JH and IgK rearrangements are screened. For suspected T cell proliferations, TCRB and TCRG rearrangements are sought.

The tests are DNA-based and can be performed on peripheral blood, bone marrow (EDTA anti-coagulated specimens) or DNA extracted from fresh or paraffin-embedded tissue biopsies.

Interpretation

The following limitations of molecular clonality studies have been recognised:

1. Limited sensitivity, related to normal polyclonal background

2. Clonality is not equivalent to malignancy
3. Ig and TCR gene rearrangements are not markers for lineage
4. Pseudoclonality and oligoclonality issues.

Consequently, such results must be integrated into the broader clinical and laboratory findings of individual patients.

Analysis of IgVH Mutational status in Chronic lymphocytic leukaemia.

The presence or absence of somatic mutations in the variable region of the immunoglobulin heavy chain gene (IgVH) has emerged as an important prognostic factor in chronic lymphocytic leukaemia (CLL). The median survival of patients with unmutated IgVH genes showing greater than 98% homology to the germ-line sequence ranges from 79 – 119 months compared to between 124 – 293 months for patients with mutated IgVH genes. Information on IgVH mutational status in CLL has particular impact in younger patients (< 65 years of age) where more intensive therapeutic options including autologous and allogeneic stem cell transplantation are under consideration.

Discussion with Molecular Laboratory staff is advised prior to requesting this test.

Analysis method

Multiplex PCR and automated direct sequencing: A rapid multiplex PCR method using genomic DNA and BIOMED-2 primers and protocols is used to determine the IgVH variantal status. The method involves the use of 6 VH framework primers combined with one JH primer. PCR products are purified and sequenced and variants are identified by comparison with the germ-line sequence using the Ig Blast database.

Results reported

For example:

IgVH mutational status in CLL: IgVH gene usage 3-21
Status: mutated (> 2% deviation from germline)

The median survival of patients with unmutated IgVH genes showing greater than 98% homology to the germ-line sequence ranges from 79 – 119 months compared to between 124 – 293 months for patients with mutated IgVH genes.

Miscellaneous Molecular Haematology Tests

For a complete range of tests offered here see Appendix 1.

The following molecular tests are performed in specialist centres and are best sent directly after discussion with the appropriate laboratory.

FIP1L1-PDGFR fusion gene in hypereosinophilic syndrome
KIT D816V variant in systemic mastocytosis
Rare bcr-abl transcripts
8p11 myeloproliferative syndromes

Professor N.C.P.Cross MA PhD FRCPath
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Salisbury District Hospital
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Tel: +(44) 1722 429080
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MRD monitoring: adult acute lymphoblastic leukaemia:

Dr Adele Fielding,
Royal Free and University College Medical
School, Rowland Hill, London NW3 2PF
Email: A. Fielding@medsch.ucl.ac.uk

Molecular analysis and family studies in inherited coagulation disorders including haemophilia A, haemophilia B, von Willebrands disease and fibrinogen disorders:

Dr Andrew Mumford,
Haemophilia Centre Director,
Dept of Haematology,
Bristol Royal Infirmary,
Bristol BS2 8HW
Tel 0117 9282597
Email Andrew.Mumford@UHBristol.nhs.uk

Appendix 1:
UHBristol SiHMDS Molecular Haematology user guide.

Contact: 0117 342 0779
Head of Department: Tim Clench.
Email: Tim.Clench@UHBristol.nhs.uk

UHB HMDS MOLECULAR GENETICS TESTS

Diagnostic query	Test	Sample required	TAT	Further information
Acute Leukaemia B	BCR-ABL	EDTA	24hrs	Contact lab if urgent
	ASO MRD - Trial	EDTA		
Acute Leukaemia T	ASO MRD - Trial	EDTA		
Acute Myeloid Leukaemia	PML-RARA	EDTA	5hrs	Contact lab
	BCR-ABL	EDTA	24hrs	Contact lab if urgent
	NPM-1	EDTA	48hrs	
	CEBPA***	EDTA	10 days	

	FLT-3 ITD	EDTA	48hrs	
	FLT-3 variant	EDTA	10 days	
	*NGS variant panel	EDTA		See below
MPN	BCR-ABL	EDTA¶(4)	10 days	
	JAK-2 V417F; exon 12***	EDTA	10 days	
	MPL***	EDTA	10 days	
	CALR***	EDTA	10 days	
	If trplneg: SF3B1, SFSR2	EDTA	20 days	
MPN - familiar/other	MPL Baltimore**	EDTA	10 days	
Eosinophilia	BCR-ABL	EDTA	10 days	
	T cell clonality	EDTA	10 days	
MDS / CMML	JAK-2	EDTA	10 days	
	SFSR3***	EDTA	10 days	
	SRSF2*** and NPM1	EDTA	10 days	
? AA ? Hypoplastic MDS	*NGS variant panel	EDTA		See below
Bone marrow failure	*NGS variant panel	EDTA		See below

CNL	CSF3R***	EDTA	10 days	
Mastocytosis	KIT***	EDTA	10 days	
T-LPD - general	T cell clonality	EDTA	10 days	
LGL	STAT3***	EDTA	10 days	
B-LPD - general	B cell clonality	EDTA	10 days	
CLL	IGVH	EDTA	15 days	
	p53, NOTCH variants by NGS*	EDTA		See below
MCL	SOX-11***	EDTA	10 days	Usually not required
LPCL / WM / ?Lymphoma in Vitreous fluids	MYD88***	EDTA	10 days	
HCL	BRAF V600E***	EDTA	10 days	

*NGS panels – see next page for full information

** MPL Baltimore: A (rare) variant that may be found in individuals (usually of African or Afro-Caribbean ethnic origin) with persistently raised platelets, that has no link with progression to neoplasia.

***Validated, not UKAS accredited.

¶If for TKI treatment monitoring, then four EDTA samples are required

Other molecular tests

Diagnostic query	Test	Sample required	TAT	Further information
Thrombophilia	Factor V Leiden	EDTA/Citrate	10 days	Please contact coagulation medical team before requesting these tests
Thrombophilia	Prothrombin 20210	EDTA/Citrate	10 days	
Pre-treatment with purine analogues	TPMT***	EDTA	10 days	
Raised ferritin with Hx. Of cataracts	HHCS***	EDTA	10 days	
?Hereditary haemocromatosis	Haemochromatosis	EDTA	10 days	