King's College Hospital

Laboratory for Molecular Haemato-oncology (LMH)

Laboratory User's Handbook
2020 version 2.3

This handbook is intended to provide users of LMH services with information required to select and request tests, collect, label and transport samples and to request or view reports and to obtain further information or help in interpreting results. It is published via web sites as a printable Portable Document Format file and is updated periodically (typically every 6 – 12 months). Service users should be aware that copies that they print or distribute may become outdated, so they are advised to check the web site periodically. If you wish to be informed when a new edition is published, please contact the LMH Manager or Quality Manager.

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1. General Information.

King’s Haematological Malignancy Diagnostic Centre

King's has been a Regional Centre for diagnostic services for over 20 years, providing Immunophenotyping, Cytogenetic and Molecular Diagnostic services to haematologists; covering most of Southeast England, with populations varying from densely urban to rural with wide variations in levels of affluence and deprivation.
There has been a steady rise in referrals (now over 12,000 samples per year) to the King's laboratories, from clinicians who require increasingly sophisticated and accurate haematological diagnoses, for diagnosis and disease monitoring.

Key features:

- The Flow Cytometry laboratory now processes over 6,000 samples per year for Immunophenotyping.
- The Cytogenetics/FISH laboratory analyses over 4,000 samples/year by conventional cytogenetics and over 2000 samples/year by FISH techniques
- LMH provides a wide repertoire of translocation detection, mutation screen analyses and quantitative monitoring for Minimal Residual Disease in myeloid and lymphoid diseases.
- Pre- and post-transplant molecular analysis with a 16 point genetic profile provides sensitive post-transplant Chimerism monitoring of engraftment to guide clinical intervention with DLI.
- With the publication of the National Institute for Clinical Excellence (NICE) guidance on the cancer services, 'Improving Outcomes in Haematological Cancers' (IOG), King's has made the logical extension to develop the Regional Specialist Diagnostic Services as outlined in the guidance.
- This is also consistent with the Carter Report recommendations on NHS laboratory services in England with regard to building capacity and providing an enhanced level of equipment and expertise. King's has appointed three full-time HMDC Consultants to develop these services and providing NICE IOG-compliant 'whole-system' integrated diagnostic and reporting processes capable of producing a final electronic integrated diagnostic report. This is achieved by developing a multidisciplinary team of specialist pathologists who provide a high-volume, rapid turn-round, integrated diagnostic service in a single centre.

We aim to provide:

- Efficient, comprehensive diagnostic services for haematological malignancies.
- An effective interface with clinical Haemato-Oncology services through multidisciplinary meetings (MDM).
- Research, development, evaluation and the introduction of new diagnostic techniques.
- Rapid response to technology changes and clinical needs.
- Support to clinical and epidemiological research.
- Education and training programmes for clinical and scientific staff

1.1 HMDC Laboratory for Molecular Haemato-Oncology at King's College Hospital Laboratory (LMH) overview

Molecular techniques of analysis are a rapidly growing and powerful tool for identifying and quantifying molecular abnormalities/mutations for diagnosis, prognosis and monitoring of haematological malignancies. Our laboratory has developed sensitive PCR techniques for detecting and quantifying molecular markers for this routine service.

Key features:

1. LMH provides a specialist diagnostic service that is fully integrated with the King’s Haematological Malignancy Diagnostic Centre (King’s HMDC). The laboratory is committed to providing an excellent service with emphasis on quality, speed, assay development and integration of new technologies and discoveries.
2. LMH has been an accredited laboratory since 2003 and was accredited by UKAS (Lab No. 9597) against the ISO 15189:2012 standards in May 2018). See https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/9597%20Medical%20Single.pdf for Schedule of Accreditation. All work is carried out within the framework of a documented quality system.
3. The laboratory participates in accredited External Quality Assessment schemes for all tests where these exist and less formal schemes or peer-exchange schemes where they do not. Any poor EQA performance or other quality assurance non-conformances are investigated and, where appropriate, analysed to determine possible effects on the quality of results and reports. Where analysis indicates a risk of clinically significant effects, LMH will communicate and co-operate with referrers and any necessary third parties to contain risks, and will implement remedial and corrective actions.
4. Information about patients and donors is held in compliance with the General Data Protection Regulations and Freedom of Information Act.
5. LMH estimates and monitors Measurement Uncertainty and other quality metrics for its tests and uses these to control and seek to improve test performance. This information, together with EQA performance data is available on request from the Laboratory or Quality Managers. Estimates of Measurement Uncertainty for tests performed on individual samples can also be provided and the laboratory, in conjunction with HMDC consultants can provide advice on interpretation of results, the limitations of tests and, where appropriate, the value of repeat analysis or repeat sampling.

1.2 Where to find LMH

Postal addresses

Sample Delivery Address	Correspondence Address
LMH c\o King's Haematological Malignancy Diagnostic Centre Viapath Central Specimen Reception Ground Floor, Bessemer Wing King's College Hospital NHS Foundation Trust Denmark Hill London SE5 9RS	Laboratory for Molecular Haemato-oncology The Rayne Institute King's College Hospital NHS Foundation Trust 123 Coldharbour Lane Camberwell London SE5 9NU

1.3 Key Personnel and Contact Details

LMH direct lines: Results Phone 020 7848 5809 Fax 020 7848 5820

Internal 772 5809 / 772 5821

Duty Scientist email contact kch-tr.LMH@nhs.net (this is the best address to use for general enquiries)

Clinical Scientist advice: Dr Nicholas Lea and Dr Aytug Kizilors

Medical advice: Clinical Lead Dr Robin Ireland in the first instance or HMDC consultants as below

LMH			
Designation	**Name**	**Telephone**	**Email**
Laboratory Lead	Dr Nicholas Lea	0207 848 5821	nlea@nhs.net
Deputy Laboratory Lead	Dr Aytug Kizilors	0207 848 5809	akizilors@nhs.net
Clinical Lead	Dr Robin Ireland	0203 299 2283	robin.ireland@nhs.net
Quality Manager	Mrs Tanya Scott	0203 299 4959	tanya.scott@nhs.net
HMDC			
Consultant Haematologist	Dr Robin Ireland	0203 299 2283	robin.ireland@nhs.net
Consultant Haematologist	Dr Shireen Kassam	0203 299 5262	shireen.kassam@nhs.net
Consultant Haematologist	Dr Debby Yallop	0203 299 1217	deborah.yallop@nhs.net

Complaints, suggestions and feedback

King's College Hospital is committed to continuously improving the quality and range of services provided and welcomes any comments or suggestions from users. Please contact the Laboratory or Quality Manager in the first instance regarding complaints and suggestions.

Complaints are managed via our Quality Management System and we always strive to provide a satisfactory response to any complaint. The complaints procedure is available from the laboratory or quality manager.

1.4 Laboratory Opening Times

The LMH laboratory is open from Monday to Friday: 9 am to 5:30 pm.

The laboratory is not open on bank holidays.

Avoid sending samples on a Friday as they may not arrive in time for sample processing and there is a risk that they may be too old to process for RNA-based tests.

2. Use of the Laboratory

2.1 Test Requesting Procedure (routine, urgent and out of hours)

Routine requests can be made either by King's EPR system (Denmark Hill site internal users) or the HMDC request form (external users). During normal laboratory hours please telephone urgent requests (020 7848 5821 / 5809) or speak to a HMDC consultant to ensure priority processing.
It is the responsibility of the test requester to ensure that patient consent has been obtained.
The web page http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre summarises the service provided by the King's HMDC and is used to publish downloadable copies of this user guide, the HMDC request form and a document (named "SNP Operational Processes") that illustrates the clinical algorithms, in flow chart format, used to indicate the circumstances under which Single Nucleotide Polymorphism Karyotyping is used to replace or supplement traditional cytogenetic analysis.

2.2 Requesting Additional Tests

Depending on the original request, the laboratory will attempt to preserve material for additional testing where required. Please contact the lab if further testing is required on a particular sample. Refer to Specimen retention policy below for the retention periods for different sample types.

2.3 Completing the Request Form

Please use labels printed from laboratory computer systems whenever possible to maximise legibility and minimise risks of patient misidentification.
The minimum required dataset for request forms and sample labels is stated below.
Please note that inadequately labelled samples or those accompanied by inadequately completed forms will not be processed under any circumstances (see Dataset: below).

EPR requests:
At the Denmark Hill site the following patient sample requests can be made via EPR. Please select the correct test (these are listed in Section 8: Additional Information on Molecular Investigations in LMH lab below) and enter free text in the clinical details field if the test is not listed.

Paper based request forms:
The HMDC Request form may be used for all requests. It is published at:
http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre
All hand written forms should be completed legibly.
Referral of samples from the EPR system or accompanied by a signed and completed HMDC request form and acceptance of this sample for testing by the laboratory constitutes an agreement between the Requester and the HMDC laboratories.

Dataset:

An EPR-based or a paper-based request must accompany all specimens sent to the laboratory. It must state the following information clearly and legibly.

The details on the sample must have at least three points of identification (i.e. surname and first name, date of birth and hospital number or NHS number) and these must be identical on the request form to link the sample and request form unequivocally and for the sample to be processed.

The minimum dataset requirement for the form is listed below.

Patient Identifying Data:
- **Surname/family name and first name(s) in full (surname and first name are one identifier)**
- **NHS number, hospital number (KCH or other hospital) or other patient unique identification number (the same number must be on both the tube and the form)**
- **Date of birth**

Sample Identifying Data:
- **Date specimen taken**

Other Essential Data Required for Acceptance
- **Type of specimen**
- **Tests required**
- **Address for report**
- **Requesting Consultant**

Other useful data
- Contact details of the requester (Email and Phone)
- All relevant clinical details including:
  - Treatment
  - Transplant date (if transplanted)

- Known molecular features Transcript type (e.g. BCR-ABL1 P210 or P190)

If uncertain about the exact test and terminology, please give a detailed clinical history as this can help HMDC & LMH personnel to decide the most appropriate investigations.

2.4 Specimen Labelling

- Use labels wherever possible. Samples must have handwritten labels unless demand-printed labels are produced at the time of phlebotomy or biopsy.
- The specimen must be labelled with the same patient details as those on the request form
- Please ensure that hand written details are legible
- Please note that unlabelled or inadequately labelled specimens cannot be processed and will be discarded.

2.5 Specimen Collection

The best results are obtained when an appropriate, well-taken specimen is in the proper container, is delivered to the laboratory promptly and relevant clinical information is provided on the request form. Please contact the laboratory if there is any doubt about the best specimen to take or you have questions about any test.

General guidance on specimen collection includes:

- Send specimens in sterile containers
- Take specimens that are representative of the disease process.
- An adequate quantity of material should be obtained for complete examination (refer to HMDC request form for required volumes)
- Sample tubes must not be opened following blood collection or used for any testing prior to being sent
- Sample tubes must be stored at room temperature or + 2 to +8 ^{o}C before transport.
- Samples MUST be labelled, dated and signed by the person taking the sample.

2.6 Specimen Limitations Affecting Assay Performance

Factors that can affect assay performance are as follows

- collection container: use of correct blood collection tubes – EDTA is required for molecular investigations
- Sample volume (this is particularly important for all RNA-based assays where volumes < 20ml may be insufficient)
- Storage and transportation conditions (see below)

2.7 Transport and packaging of Specimens (External Customers)

It is the responsibility of the sending organisation to ensure that samples are packaged in accordance with the current European Agreement concerning Carriage of Dangerous Goods by Road Regulations (packaging instructions 650) to prevent breakage or spillage in transit. The outside of the box or package containing the samples must be clearly addressed and the package must be accompanied by a correctly completed request form. Courier / taxi / suitable transport should be arranged by the sending institution or laboratory and the sender is responsible for ensuring the contractor can guarantee safe, timely and controlled transport and accurate delivery.

Samples must reach the laboratory in time to be processed during laboratory working hours within set time limits after venepuncture. Sample reception is open 24/7 but samples must arrive so that they can be processed within the time limits below.

First class postage is adequate but samples must be shipped with packaging appropriate for UN 3373 samples following packing instruction 650. See http://www.royalmail.com/sites/default/files/Guidance-Document-Infectious-Substances-171012.pdf for further details: Samples must be transported in a way that protects their integrity and must not be exposed to extremes of temperature (> 25^{o}C) or prolonged transport (> 2 days). **This is particularly important for RNA-based tests and referrers are advised to use approved courier services that guarantee delivery times and controlled temperature transport for these tests**. If samples are in danger of being exposed to conditions where sample integrity or quality could be compromised or if further advice is needed, please contact the laboratory to discuss the most appropriate method of transport.

2.8 Transport and packaging of specimens (internal customers)

ROUTINE SAMPLES:

All King's specimens should be taken to central specimen reception at King's College Hospital or sent via the Pneumatic Air Tube Transport System (PATTS).

However, any difficult to resample or unrepeatable specimen of any type must not be sent via the air tube:

URGENT SAMPLES:

For all testing, send or bring the specimen **DIRECTLY** to Central Specimen Reception on the ground floor of the Bessemer Wing. *Please telephone 020 7848 5821 / 5809 for specimens to be processed urgently*.

2.9 High Risk Specimens and Safety

The LMH lab is currently not able to process the following sample types due to the risk from Covid-19: buccal lavage, saliva, sputum.
Samples from patients with suspected viral haemorrhagic fevers or with a history of having returned within 21 days from Africa, Asia and South America are considered high risk. Contact Virology medical staff before taking ANY sample. LMH is unable to accept samples of this nature as they do not have the facilities to deal with them.

Great care must be taken in obtaining specimens. Equipment such as needles and blades must be immediately disposed of safely in locally approved "sharps" bins and NOT SENT TO LABORATORIES. Specimens should be transported to the laboratory without delay.

2.10 Result Availability

Our target is to issue reports from date of sample receipt within the intervals listed in sections 4, 5 & 6 below. Urgent samples may be tested more quickly by prior arrangement with the laboratory but turnaround times are limited by factors intrinsic to analytic procedures and prioritisation will always be according to clinical criteria. The requester will be notified by email or telephone if a significant delay in reporting is anticipated. Reports will be sent to the address indicated on the referral form and reports are also available on the Results Online reporting system.

Exporting of reports:

- LMH laboratory reports are exported to the HMDC integrated reporting system immediately after authorisation.
- Integrated HMDC reports are dispatched only after the results of all investigations have been completed and authorised by an HMDC consultant. Where a report is required urgently or if an outstanding investigation will significantly delay reporting, an interim report is released by the HMDC clinical team.
- For investigations requested by EPR a copy of the HMDC integrated report is associated with the EPR record.
- External referrals are reported by printing and mailing to the address provided by the requester. Results are also available through the Results Online service.
- Copies of printed reports can be obtained by telephoning extension 020 3299 5862.

2.11 Specimen Retention Policy

Specimens are retained by the laboratory in order to repeat analysis or to enable additional analysis to be performed. The laboratory will attempt to retain / preserve samples in such a way to allow retesting and additional testing based on the clinical information provided. All samples are retained for a minimum of 5 years. Control material stored to facilitate the analysis of chimerism will be stored for a minimum of 10 years.

2.12 Telephoned Results

- Results are not routinely telephoned but HMDC clinical staff may contact requesters / care providers where considered clinical relevant

2.13 Visitors

Visitors should introduce themselves at the Rayne Reception Ground Floor Rayne Institute, Coldharbour Lane. Security staff will call the laboratory to obtain the person they wish to see, who will come to meet them. It is best to make appointments in advance to ensure the right person is available.

3. Out of Hours' Service

3.1 Out of Hours' Examinations Provided in LMH

No on-call service is currently provided by LMH. Laboratory opening times are 09:00 to 17:30 Monday to Friday,

3.2 Medical Advice

During weekdays from 9 am to 5:30 pm medical advice on interpretation of LMH results, or advice about clinical management, or any other relevant clinical circumstance can be sought from the HMDC consultants see section 1.3.
For out of hours advice (urgent clinical situations only), contact the haematology Specialist Registrar bleep holder via the KCH switchboard on 020 3299 9000

4. List of Tests Performed in LMH

Molecular Investigations			
Test	**Specimen type**	**Schedule**	**Turnaround Time****
BCR-ABL1 major (P210) quantitation*	EDTA PB or BM	Daily (week days)	5 days
BCR-ABL1 minor (P190) quantitation*	EDTA PB or BM	On demand	5 days
BCR-ABL1 Multiplex*	EDTA PB or BM	Twice weekly	5 days
PML-RARA translocation quantitation*	EDTA PB or BM	On demand	7 days
RUNX-RUNXT1 translocation quantitation*	EDTA PB or BM	On demand	7 days
CBFB-MYH11 translocation quantitation*	EDTA PB or BM	On demand	7 days
Post SCT Chimerism analysis	EDTA PB or BM	Twice weekly	5 days
JAK2 V617F mutation quantitation	EDTA PB or BM	Daily (week days)	5 days
JAK2 exon 12 mutation detection	EDTA PB or BM	Daily (week days)	5 days
CALR mutation detection	EDTA PB or BM	Daily (week days)	5 days
MPL W515 mutation detection	EDTA PB or BM	Daily (week days)	5 days
BRAF V600E mutation detection	EDTA PB or BM	Weekly	10 days
MYD88 L265P mutation detection	EDTA PB or BM	Weekly	10 days
ABL1 kinase domain mutation detection*	EDTA PB or BM	Every two weeks	30 days
Myeloid gene panel#	EDTA PB or BM	Every two weeks	30 days
SNP array karyotyping	EDTA PB or BM	Weekly	15 days
FLT3 / NPM1mutation analysis	EDTA PB or BM	On demand	3-5 days
TP53, SF3B1, RUNX1	EDTA PB or BM	Every two weeks	30 days

***** These tests utilise RT qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the Section 8: Additional Information on Molecular Investigations in LMH lab below for further information.

** Maximum turn round time in working days from receipt of sample during working week

MGP analysis is currently performed by a method outside of the laboratory scope of UKAS accreditation. This is a temporary situation and will be added back to the laboratory scope in the near future.

5. Tests Referred to Other Laboratories

Test	Sample type	Reference Laboratory	Turnaround Time
KIT mutation detection and monitoring*	EDTA BM only	Wessex Regional Genetic Laboratory	20 days
FIP1L1-PDGFRA translocation* (monitoring only)	EDTA PB or BM	Wessex Regional Genetic Laboratory	20 days
NPM1 transcript monitoring**	EDTA PB or BM	Viapath Molecular Oncology Unit, Guys Cancer Centre UKAS Lab No 8688	14 days
IgVH resequencing**	EDTA PB or BM	Leeds HMDS	21 days

Key:
***** = NOT a test within the ISO15189 accreditation scope of the laboratory
** = a test within the ISO15189 accreditation scope of the sendaway laboratory

6. External Quality Assurance (EQA) scheme participation

Test	EQA Schemes
BCR-ABL1 major (P210) quantitation	NEQAS
PML-RARA translocation	NEQAS
RUNX-RUNXT1 translocation	NEQAS
CBFB-MYH11 translocation	NEQAS
Post Stem Cell Transplant Chimerism analysis	NEQAS
JAK2 V617F mutation quantitation	NEQAS
JAK2 exon 12 mutation detection	NEQAS pilot scheme
CALR mutation detection	NEQAS pilot scheme
MPL W515 mutation detection	NEQAS pilot scheme
BRAF V600E mutation detection	NEQAS
MYD88 L265P mutation detection	NEQAS pilot scheme
ABL1 kinase domain mutation detection	NEQAS
Myeloid gene panel	NEQAS pilot scheme
SNP array karyotyping	CEQAS
Amplicon sequencing of TP53 (NGS)	ERIC EQA scheme

7. Additional Information on Molecular Investigations in LMH lab

7.1 CALR Mutations

Somatic JAK2 V617F mutation is present in the majority (95%) of patients with Polycythaemia Vera (PV), but only in 50-60% of patients with Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). Mutations in the gene encoding thrombopoietin receptor (MPL) exist in a small subset (5-10%) of patients with JAK2 V617F negative ET and PMF.

Recently, recurrent mutations in the gene encoding Calreticulin (CALR) have been identified in a majority (70-85%) of MPN patients without JAK2 or MPL mutations. CALR mutations are never seen in PV and are always mutually exclusive to JAK2 and MPL mutations. These heterozygous mutations (insertions or deletions), located in exon 9, lead to a frameshift to a specific altered reading frame and result in a mutant protein with a novel C-terminal. ET and PMF patients with CALR mutations have lower haemoglobin, higher platelet counts, lower risk of thrombosis and a better overall survival than patients with mutated JAK2.

The causal relationship between CALR mutations and excessive platelet production is further supported by identification of these mutations in a proportion of RARS-T patients but not in MDS, AML or CML. Thus, CALR mutations are the second most frequent mutation after JAK2 in MPN's and impart a more indolent phenotype. CALR mutations are major driver events in the pathogenesis of MPN, occurring at the stem cell level and remain stable during disease evolution.

We suggest sequential testing on peripheral blood in order of mutation frequency for 1) JAK2, 2) CALR and 3) MPL to improve diagnostic accuracy and efficiency of myeloproliferative neoplasms in the same way JAK2 testing has in the last decade.

Mutation Testing: CALR mutations are detected using a PCR fragment analysis approach which has a sensitivity of around 5%

Factors affecting results or interpretation: Samples received in heparin are not amenable to PCR based analysis and can therefore not be tested for JAK2 mutations.

7.2 JAK2 & MPL Mutations

Background:

In 2005 a novel somatic point mutation (V617F) in the conserved autoinhibitory pseudokinase domain of the Janus kinase 2 (JAK2) protein (encoded on chromosome 9p24) was described. This mutation is present in patients with myeloproliferative neoplasms at various frequencies (PV=97%, ET=50% and MF=50%). Since the discovery of JAK2 V617F as a clonal marker of MPN several other mutations have been described in this patient group and occur at differing frequencies. Notably mutations of JAK2 exon 12 and mutations of MPL at codon 515. JAK2 exon 12 mutations appear to be restricted to cases of PV, almost no cases of ET or MF have been described harbouring JAK2 exon 12 mutations. Conversely MPL mutations are restricted to ET and MF and are not been described in PV patients.

JAK2 Mutation Analysis at King's College Hospital:

KCH has a long-standing research interest in MPD. We were the first to show that JAK2 mutations occur in about 50% of Budd Chiari Syndrome patients (Patel et al 2006) and have published a number of articles documenting the incidence of JAK2 mutations in various haematological malignancies (Lea et al 2006, Ingram et al 2006 and Ceesay et al 2006).

Mutation Testing:

JAK2 V617F mutations are detected using a quantitative real time PCR (Q-PCR) based assay in the LMH laboratory which has a sensitivity of around 0.2%.

MPL W515K/L mutations are detected using a sensitive allele specific PCR reaction with a sensitivity around 1% JAK2 exon 12 mutations are detected using a fragment analysis and allele specific PCR approach with a sensitivity around 1% Due to the rarity of MPL and JAK2 exon 12 mutations these laboratory tests are not normally performed without a previous JAK2V617F negative result and a strong suspicion of an MPN. Exon 12 and MPL assays can be performed on the original JAK2V617F test DNA sample. It is not necessary to send an additional sample to perform these analyses. Please contact the laboratory to arrange this additional analysis.

Factors affecting results or interpretation: Samples received in heparin are not amenable to PCR based analysis and can therefore not be tested for JAK2 or MPL mutations. Note there are rare mutations of both JAK2 and MPL occurring in other codons that are not detected using with the assays deployed by the LMH laboratory.

7.3 FLT3 & NPM1 mutation analysis

Clinical Relevance / Purpose

1. An internal tandem duplication in the fms-like tyrosine kinase 3 gene (FLT3/ITD) is a common finding in approximately 25% of younger adult AML patients in acute myeloid leukaemia (AML) (Small 2006). The length of the ITD varies between 3 and 400 bases and is always in frame and therefore expected to produce functional protein.

2. Point mutations that most frequently involve aspartic acid 835 of the tyrosine kinase domain (TKD) are the second major type of FLT3 mutations, seen in 8-12% of patients with AML (Small 2006). FLT3-TKD point mutations, along with deletions and insertions, have also been found in several other sites (Patnaik MM 2018).

3. In vitro studies have shown that insertions lead to a constitutively activated receptor (Hayakawa F et al 2000). It is widely accepted that, in AML patients, FLT3-ITDs are associated with leucocytosis, a high percentage of bone marrow blast cells, increased risk of relapse from complete remission (CR) and reduced survival (Kottaridis PD et al 2003). FLT3-ITD is an uncommon mutation in MDS (3-6%) but its presence is linked with a high probability of MDS transforming to AML (Pinheiro RF et al 2008).

4. FLT3-TKD mutations often involve the activation loop, and thus also result in constitutive activation of the receptor's tyrosine kinase activity (Small 2006). FLT3-TKD mutations have been associated with high percentages of peripheral blood and bone marrow blast cells, but the prognostic impact is still unclear. (Frohling S et al 2002).

5. The 2017 ELN recommendations include molecular testing for both FLT3-ITD and FLT3-TKD mutations, due to the emergence of FLT3 inhibitors, such as midostaurin, in treatment of patients with FLT3-mutated AML (Dohner H et al 2017).

6. Mutations in exon 12 of the nucleophosmin (NPM) 1 gene have been reported in AML and MDS patients with normal karyotype. They take the form of a 4 bp insertion. Overall NPM1 mutations generally are associated with a trend for improved overall survival and significantly better event free or relapse-free survival, and in multivariate analysis they are an independent good prognostic factor for outcome (Schnittger S et al 2005)

7. The favourable outcome associated with NPM1 mutations is largely lost in the presence of a FLT3/ITD (Gale R et al 2008)

Principle

1. 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. Mutated genes give rise to larger PCR amplicons and wild type genes produce an amplicon of a predictable and stable size.

2. 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

3. To determine the FLT3 TKD mutation status the primers are positioned to target TKD mutations in the activating loop of the kinase domain. Wild type FLT3 gene includes an EcoRV restriction digest site. When nucleic acid substitution occurs, as with FLT3-TKD mutations, the restriction digest recognition site disappears. In order to identify the FLT3-TKD point mutation using fragment length analysis, an EcoRV digest is performed on PCR amplified target region of FLT3. One of the PCR primers contains an EcoRV restriction site, allowing both wild type and mutant genes to be digested with different digestion patterns.

4. 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. This is relevant because high allele burden for FLT3-ITD gives a worse prognosis than low allele burden (Gale R et al 2008).

Note the FLT3 TKD mutation detection aspect of this assay is currently outside of the laboratories scope of UKAS accreditation.

7.4 Myeloid Gene Panel

Purpose of the test: the Myeloid Gene Panel (MGP) uses Next-Generation Sequencing (NGS) technology to identify somatic mutations in myeloid malignancies for patient diagnosis and/or prognosis. The panel is designed to sequence genes or gene sub-regions (hot spots) that are frequently mutated in Acute Myeloid Leukaemia (AML), Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPN). The panel provides a comprehensive assessment of the key genes involved in myeloid malignancies in a single test. The panel is not designed for MRD monitoring as the limit of detection is set at 5% variant allele frequency.

Sample requirements: this method is designed to detect somatic and inherited mutations in clinically relevant sample, for example peripheral blood, bone marrow and blood films can be used. Fresh samples should be provided in EDTA or Heparin. The use of DNA from fixed cells is currently undergoing validation. If DNA is provided from an external user, the DNA should be between 10-50 ng/µl. It is possible to use less than 10ng/µl, but this is not recommended, as gene coverage will be compromised and variants may be missed.

Methods and validation: we use the QiaSeq Custom DNA Amplicon methodology from Qiagen. This involves DNA fragmentation, ligation of indexing and sequencing adapters, target enrichment and PCR amplification. Libraries are sequencing on an Illumina NextSeq 550 instrument. The library preparation may be performed manually or using a Hamilton Microlab Star liquid handling system. The reaction chemistry uses Unique Molecular Indices (UMIs) to reduce background artefacts. QIAseq Targeted DNA Panels have also been optimized in combination with a specially formulated enrichment chemistry to achieve highly efficient enrichment on both regular and GC-rich regions at high multiplex levels. In-house data analysis is performed using our validated bioinformatics pipeline (Snappy) and variant calling and analysis software (SQVD and VASA).

Factors affecting results or interpretation: This method has undergone thorough validation and an extension to scope is in preparation under our existing UKAS ISO15189 accreditation. The sensitivity of the MGP assay is limited to 5% variant allele frequency if target coverage is >400x. If coverage is between 200-400x, limit of detection is 10%. This panel is ideally suited to the detected of SNVs and small insertion/deletions. Insertions of more than 100bp may not be detected, as is the case for large FLT3-ITDs. This assay is not suitable for monitoring patients post treatment, including post transplantation, unless relapse is suspected.

The MGP targets are as follows:

Gene	Chr	Targets	Transcript
ANKRD26	chr10	all coding	NM_014915
ASXL1	chr20	12	NM_015338
BCOR	chrX	all coding	NM_001123385
CALR	chr19	9	NM_004343
CBL	chr11	7 + 8 + 9	NM_005188
CEBPA	chr19	all coding	NM_004364
CHEK2	chr22	all coding	NM_001005735
CSF3R	chr1	13 - 18	NM_156039
CUX1	chr7	all coding	NM_181552
DDX41	chr5	all coding	NM_016222
DNMT3A	chr2	all coding	NM_022552
ETV6	chr12	all coding	NM_001987
EZH2	chr7	all coding	NM_004456
FLT3	chr13	14 + 15 + 20	NM_004119
GATA1	chrX	all coding	NM_002049
GATA2	chr3	all coding	NM_032638
HRAS	chr11	2 + 3	NM_001130442
IDH1	chr2	4	NM_005896
IDH2	chr15	4	NM_002168
IKZF1	chr7	all coding	NM_006060
JAK2	chr9	12 + 14	NM_004972
KIT	chr4	2, 8-11, 13 + 17	NM_000222
KMT2A	chr11	all coding	NM_001197104
KRAS	chr12	2 + 3	NM_033360
MPL	chr1	10	NM_005373
NF1	chr17	all coding	NM_001042492
NFE2	chr12	all coding	NM_001136023
NPM1	chr5	12	NM_002520
NRAS	chr1	2 + 3	NM_002524
PHF6	chrX	all coding	NM_032458
PPM1D	chr17	all coding	NM_003620
PTPN11	chr12	3 + 13	NM_002834
RAD21	chr8	all coding	NM_006265
RUNX1	chr21	all coding	NM_001754
SETBP1	chr18	4	NM_015559
SF3B1	chr2	12 - 16	NM_012433

SH2B3	chr12	all coding	NM_005475
SRSF2	chr17	1	NM_003016
STAG2	chrX	all coding	NM_001042749
TET2	chr4	all coding	NM_001127208
TP53	chr17	all coding	NM_000546
U2AF1	chr21	2 + 6	NM_006758
WT1	chr11	7 + 9	NM_024426
ZRSR2	chrX	all coding	NM_005089

7.5 BRAF V600 Mutation

BRAF is the most commonly mutated gene in human cancers and encodes a serine/threonine protein kinase involved in cellular growth signalling (Davies et al., 2002). In Hairy Cell Leukaemia (HCL), the V600E variation has been found in a high proportion of cases (see Tiacci et al., 2011) proving to be an effective diagnostic marker for HCL. Early diagnosis of HCL is important because it can translate into a treatable disease outcome using highly effective purine nucleoside analogues (Grever, 2010).

The purpose of this assay is the screening of the V600E and V600K variation in BRAF using an allele specific PCR-based Amplification Refractory Mutation System (ARM) to aid pathologists in the diagnosis of HCL in ambiguous cases.

References:

Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417:949-954

Ellison G, Donald E, McWalter G, et al. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. J Exp Clin Cancer Res 2010; 29:132.

Grever MR. How I treat hairy cell leukemia. Blood 2010; 115:21-28

Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. New England Journal of Medicine 2011; 364:2305–2315.

Factors affecting results or interpretation:
The sensitivity of the BRAF V600 assay is limited to 0.5% variant allele frequency. Samples containing lower concentrations of tumour cells are likely to subject to false negative results. This assay may be suitable for monitoring patients post treatment including post transplantation.

7.6 MYD88 L265P Mutation

MYD88 L265P is a mutation present in the B-cell lymphoproliferative disorders Waldenstrom's Macroglobulinemia (WM) and IgM Monoclonal Gammopathy of Undetermined Significance (IgM MGUS). MYD88 L265P has been found in other B-cell disorders at a lower level, including Diffuse Large B-cell Lymphoma of the ABC subtype (ABC-DLBCL), Splenic Marginal Zone Lymphoma (SMZL) etc. In particular this mutation was present in WM at 93% and has consistently been shown to be reported at around this level by several other groups. Studies have shown that MYD88 L265P detection in IGM MGUS patients (54%) is an early oncogenic event in the progression to WM. MYD88 was found to be a component in the process of IκBα phosphorylation and NF-κB activation by directing the construction of a signalling complex. In NF-κB signalling we can see MYD88 is integral to the pathway as it is directly or indirectly activated by Interleukin-1 receptor or Toll-like receptor 4. This triggers autophosphorylation of IRAK (intereukin-1 receptor-associated kinase) 4. At the end of this pathway IκBα is phosphorylated and NF-κB p65 and p50 is released which induces prosurvival signalling in these cells.

A PCR based assay for the MYD88 L265P mutation has been established and is based on the allele specific qPCR method published by Xu et al 2013. This PCR assay used within the LMH laboratory is capable of identifying patients who carry a mutant copy of the MYD88 allele at codon 265 at 1% mutation. This change in nucleotide sequence produces a substitution of the amino acid leucine to proline. A negative result does not rule out one of the above conditions; however a positive result would strongly suggest the diagnosis of Waldenstrom's Macroglobulinemia, and is useful in diagnosis when analysed with other clinical features. Detection of MYD88 L265P may also be useful with respects to implementing treatment strategies.

Factors affecting results or interpretation: The sensitivity of the MYD L265P assay is limited to 1% variant allele frequency. Samples containing lower concentrations of tumour cells are likely to subject to false negative results. This assay may be suitable for monitoring patients post treatment including following post transplantation.

7.7 SF3B1, RUNX1 & TP53 mutation screening by amplicon next generation sequencing

A number of mutations have been discovered which give diagnostic or prognostic information about haematological malignancies. The use of next generation sequencing is used to analyse the mutation status of these genes in several cases. Amplicon based Next Generation Sequencing offers a single generic method to analyse gene mutations and is now regarded as standard for mutation detection. Whilst the methods employed to perform sequencing in this procedure are generic to all amplicons or genes each gene requires a specific set of primers for amplification. In some cases the requirements for data analysis and interpretation may also differ.

SF3B1 In humans, the majority of all protein-coding transcripts contain introns that are removed by mRNA splicing carried out by spliceosomes. Mutations in the spliceosome machinery have recently been identified using whole-exome/genome technologies in myelodysplastic syndromes (MDS) and Chronic Lymphocytic Leukaemia (CLL). Alterations in splicing factor 3 subunit B1 (SF3B1) were the first spliceosome mutations described, immediately followed by identification of other splicing factor mutations, including U2 small nuclear RNA auxiliary factor 1 (U2AF1) and serine arginine-rich splicing factor 2 (SRSF2). SF3B1/U2AF1/SRSF2 mutations occur at varying frequencies in different disease subtypes, each contributing to differences in survival outcomes.

In myeloid neoplasms mutations of SF3B1 are strongly associated with ringed sideroblasts the frequency of these mutations in RARS/RARS-T is 68-81%. Lower mutation rates of 0-7% are observed in MDS, MDS/MPN patients with ring sideroblasts (RS) <15%. The data are slightly contentious however it seems that SF3B1 mutations are associated with a better outcome and a reduced incidence of transformation to AML in MDS.

In CLL SF3B1 mutations have also been observed at a rate of between 5 and 18% depending on the cohort studied. SF3B1 mutation is associated with rapid disease progression and shorter survival in CLL, which can be used as a prognostic marker to improve the prediction of disease progression. In CLL, SF3B1 mutations are mostly subclonal events, and therefore likely involved in disease progression.

This test is designed to detect mutations in the gene SF3B1 in patients presenting with MDS or CLL. Sequencing is restricted to the C terminal end of the SF3B1 gene where >%90 of mutations have been previously reported. Amplification is performed form genomic DNA.

RUNX1 The RUNX1 gene is the most frequent target for chromosomal translocation in leukaemia. In addition, recent studies have demonstrated point mutations in the RUNX1 gene as another mode of genetic alteration in development of leukaemia. Monoallelic germline mutations in RUNX1 result in familial platelet disorder predisposed to acute myelogenous leukaemia (FPD/AML). Sporadic point mutations are frequently found in three leukaemia entities: AML M0 subtype, MDS-AML, and secondary (therapy-related) MDS/AML. Therapy-related leukaemia resulting from anticancer treatments is not uncommon, and the incidence of RUNX1 point mutations appears comparable to the incidence of the t(8;21) AML M2 subtype and the inv(16) AML M4Eo subtype. Half of the point mutations in M0 cases are biallelic, although the frequency varies with ethnicity. Most of the RUNX1 mutations are clustered in the Runt domain and result in defective DNA binding but active -subunit binding, which is consistent with three-dimensional structural findings and may explain the dominant inhibitory effects. Unlike the classical tumour suppressor genes requiring biallelic inactivation, haplo-insufficient RUNX1 is apparently leukaemogenic. However, RUNX1 abnormalities per se are insufficient to cause full-blown leukaemia. Intensive investigation of cooperating genetic alterations should elucidate leukemic mechanisms. Principle

This test is designed to detect mutations in the gene RUNX1 (exon3-exon8) in patients presenting with transforming MDS and AML.

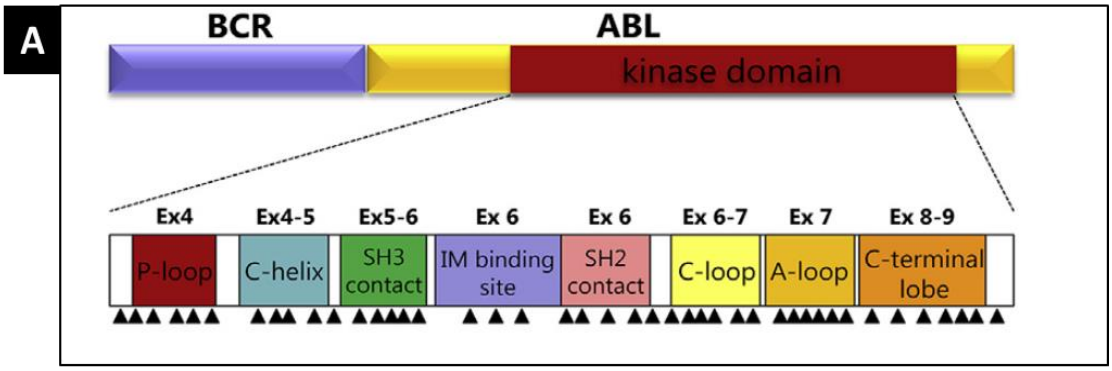
TP53 The tumour suppressor TP53 has a crucial role in cellular response to stress or DNA damage by induction of cell-cycle arrest, apoptosis or senescence. Mutational status of immunoglobulin heavy-chain variable region (IGHV) and the TP53 gene have been the most important prognostic molecular markers in chronic lymphocytic leukaemia (CLL) for a long time. Altered p53 function because of 17p deletion and/or TP53 gene mutation is associated with poor prognosis in chronic lymphocytic leukaemia (CLL) patients. Aberrations of TP53 gene occur on average in 10-15% of untreated CLL patients, but the incidence rises to 40-50% with Fludarabin-refractory CLL. It has been shown that, mutation in TP53 can also emerge during the clinical course of CLL suggesting the need for repeat testing of patients particularly when there is a change in disease status e.g. progression. It has been shown that in AML, TP53 mutation is associated with very unfavourable outcome, and TP53 mutation is much more frequent in complex karyotype AML (figure B below)). The most common TP53 mutation types in AML are missense, followed by frame-shift, splice-site, nonsense and in-frame mutations

Factors affecting results or interpretation: The sensitivity of these amplicon NGS assays is limited to 5-10% variant allele frequency. Samples containing lower concentrations of tumour cells are likely to subject to false negative results. This test is not suitable for monitoring patients post treatment including post transplantation unless relapse is suspected.

7.8 ABL1 kinase domain mutation

Imatinib or the second and third generation tyrosine kinase inhibitors (TKIs) can induce durable responses in the majority of patients with chronic myeloid leukaemia (CML) or Ph+ Acute lymphoblastic leukaemia (Ph+ALL); but some patients either fail to respond (primary resistance) or respond initially or then lose their response (secondary resistance). Although TKI resistance can be multi-factorial (BCR-ABL kinase domain (KD) mutations, BCR-ABL amplification, overexpression and clonal evolution with activation of additional oncogenic pathways), point mutations within the KD of BCR-ABL1 constitute the most frequent mechanism.

In particular, these mutations cause amino acid substitutions inside the KD, consequently impairing TKI binding and resulting in a loss of sensitivity to TKI by a direct or indirect mechanism. Depending on the regions where they are located (Figure 1), mutations can actually interrupt critical contact points between the TKI and BCR-ABL1 protein, or they can induce a conformational change, resulting in a protein to which TKI is unable to bind. At present, approximately 90 different BCR-ABL1 KD mutations have been identified. Many of these are relatively rare, whereas the most common, which account for 60-70% of all the mutations are shown in the diagram below.



B

First generation						Second generation			Third generation
imatinib						nilotinib	dasatinib	bosutinib	ponatinib
M237V	L273M	F311L	E355D/G	V379I	A397P	Y253F/H	V299L	E255K	?
M244V	E275K/Q	**T315I**	F359V/I/C	A380T	S417F/Y	E255K/V	**T315I**	V299L	
L248R	D276G	F317L/V/I/C	D363Y	F382L	I418S/V	**T315I**	T315A	**T315I**	
G250E/R	T277A	F359V/I/C	L364I	L384M	S438C	F359V/I/C	F317L/V/I/C	?	
Q252R/H	E279K	Y342H	A365V	L387M/F	E453G/K				
Y253F/H	V280A/I	M343T	L370P	M388L	E459K/V				
E255K/V	V289A	A344V	V371A	Y393C	P480L				
E258D	V299L	M351T	E373K	H396R/P	F486S				

BCR-ABL1 Kinase domain and mutations (A) Magnification of the BCR-ABL1 kinase domain, showing the main functional subdomains. Black triangles indicate Imatinib-resistant mutations. (B) Summary of the mutations reported to be insensitive to Imatinib, Nilotinib, Dasatinib, Bosutinib, and Ponatinib.

The LMH laboratory has developed Sanger and NGS sequencing approaches to interrogate the Kinase domain of BCR-ABL1. The later has an enhanced sensitivity of around 3% and is capable of detecting low level drug resistant clones early.

Factors affecting results or interpretation:

Like many translocation assays sequencing of the ABL1 kinase domain from BCR-ABL is performed from RNA which is very susceptible to degradation.

Samples received in heparin are not amenable to PCR based analysis and will not be tested.

7.9 Chimerism

The Chimerism investigation is carried out to monitor the engraftment of Bone Marrow (BM), Peripheral Blood (PB), CD3 T-cells, CD15 Granulocytes and CD19 B-Cells following Haematopoietic Stem Cell Transplant. The microsatellite regions which the individual primer sets are designed to amplify, consist of repetitive sequences (short tandem repeats STRs) of DNA 3-7 base pairs in length. These STRs are well distributed throughout the human genome and are highly polymorphic. There are many advantages of STR typing. It is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500bp long. STR typing is also amenable to a variety of rapid DNA purification techniques, which are compatible with PCR but do not provide enough DNA of appropriate quality for Southern blot-based analyses. Donor and Pre-transplant recipient samples must be obtained before the Post-transplant sample can be analysed. The donor and recipient samples are run in conjunction with all post-transplant specimens (BM, PB or fractionated cells) to illustrate the individual polymorphic alleles which distinguish the donor population from the recipient. Analysis of chimerism is valuable in assessing patient's response to transplantation. Observing a complete donor profile in the post-transplant samples indicates a successful transplant. Chimeric profiles, (the presence of both donor and recipient allelic peaks) depending on the donor to recipient ratio (%), may indicate a less successful engraftment or progression to relapse. Following analysis, donor and recipient profiles can be semi-quantified in the post-transplant sample by obtaining the values for the areas underneath respective peaks and calculating the percentages of each. A value is obtained for each of the informative genes and the mean is calculated to give a more accurate indication of the recipient status. The 16 gene profiles for each patient are printed and handed to the consultant haematologist at least 24 hours before the weekly chimerism meeting, where the individual cases are discussed with the BMT co-ordination team.

Factors affecting results or interpretation: Post-transplanted samples can only be assessed alongside a donor and pre-transplant DNA profile. These samples must be provided as a one-off prior to the sending of post-transplant material.

Reference: Edwards, A. et al. (1991) DNA typing with trimeric and tetrameric tandem repeats; polymorphic loci, detection systems and population genetics. In: The Second International Symposium on Human Identification 1991, Promega Corporation, 31 - 52. Edwards, A. et al. (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am Journal Hum. Genetics. 49, 746 – 56 Warne, D. et al. (1991) Tetranucleotide repeat polymorphism at the human B-actin related pseudogene 2 detected using the polymerase chain reaction. Nucl Acids Res. 19, 6980

7.10 SNP-Array Karyotyping

Leukaemias are characterized by recurring chromosomal and genetic abnormalities and identification of these abnormalities plays important role in diagnosis, risk assessment and patient classification as defined in the WHO classification. Current methods like metaphase cytogenetic analysis and Florescent In Situ Hybridization (FISH) to detect genomic aberrations provide limited data due to the poor in vitro growth of the abnormal clone, poor resolution and are limited to only known regions of the genome.

High-resolution Single Nucleotide Polymorphism (SNP) microarrays can identify genome-wide cytogenetically cryptic genomic aberrations that can be of diagnostic and prognostic significance. With an intermarker spacing of 750bp, SNP arrays enable very high resolution genome wide detection of copy number abnormalities. The ability to utilise a variety of clinical tissue including archived tissue material without in vitro processing, like cell culture and stimulation, provides the added benefit of unbiased cell analysis and reduced failure rate. SNP array also has the advantage of simultaneous genotyping, enabling detection of copy number neutral loss of heterozygosity (CN-LOH) or uniparental disomy (UPD). These regions have been shown to harbour point mutations in genes known to have pathogenic consequences in myeloid malignancies, such as TET2, JAK2, EZH2, CBL etc. Presence of UPD has also shown to have similar consequences on patient outcome as if they had deletions. Data interpretation is objective based on definite probe signals rather than subjective visual assessment of band intensities.

The purpose of this assay is to detect genomic aberrations in the genomic DNA from patients with haematological malignancies so as to aid in the diagnosis and treatment management of these diseases.

CytoScan Assay provides genome-wide coverage for the detection of chromosomal imbalances. The CytoScan array contains approximately 2.7 million markers which are representative of DNA sequences distributed throughout the genome with spacing, on average, approximately 880 bases apart in genic regions, and approximately 1700 bases apart in non-genic regions. The majority of the markers (1.9 million) are non-polymorphic markers, which provide overall genomic coverage of relevant cytogenetic regions and are used for assessing copy number. The assay utilises 750,000 SNPs that are DNA variants occurring in a large proportion of the human population (>1%). Each individual inherits one allele copy from each parent, so that the individual genotype at an SNP site is either AA, BB, or AB. The array contains oligonucleotides that are specific for each of the two SNP alleles, referred to as A and B. As a fluorescent signal is obtained for each allele at a given SNP site, the strength of the hybridization intensity provides information about both SNP genotype (A, B, or AB) and

copy number state (heterozygous or homozygous due to hybridization of genomic DNA to both or one probe variant, respectively).

ChAS software is used to analyse and visualize microarray data. The signal intensity of the hybridized DNA from the patient sample is compared to a reference DNA, which is based on an average of over 400 samples. The ratio of patient sample to reference intensity is expressed as a log2 ratio, and represents the relative intensity for each marker. A discrete copy number value is computed from the relative intensity data, and is displayed as the marker copy number state. The non-integer copy number states are calculated and displayed as the smoothed signal track, which can be used to support an interpretation of a mosaic gain or loss. The SNP marker A- and B-allele intensities are also visualised in the Allele Track, which can be used to confirm copy number variation regions. The allele tracks show 3 bands (AA, AB, BB) in normal diploid regions, 4 bands (AAA, AAB, ABB, BBB) in triploid regions, and 2 bands (A, B) in haploid regions. The SNP markers are also analysed for long contiguous stretches of homozygosity, which are visualized in the loss of heterozygosity (LOH) track. The absence or loss of heterozygosity (LOH) is calculated as a region significantly devoid of heterozygous genotype calls.

Factors affecting results or interpretation: The procedure relies on the availability of good quality genomic DNA obtained from the peripheral blood, bone marrow of patients the sensitivity is limited to around 20%. Samples containing lower concentrations of tumour cells are likely to subject to false negative results. This assay is not suitable for monitoring patients post treatment including post transplantation unless relapse is suspected or the tumour burden remains high. SNP-array is only capable of detecting copy number imbalances. Balanced translocations are not detected SNP-array.

Please refer to the document (named “SNP Operational Processes”) that illustrates the clinical algorithms, in flow chart format, used to indicate the circumstances under which Single Nucleotide Polymorphism Karyotyping is used to replace or supplement traditional cytogenetic analysis. This is published on the web page at http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre. It may also be accessed via this link http://www.viapath.co.uk/sites/default/files/upload/SNP%20Operational%20Processes%20Ed%202-0.pdf

Please contact the Clinical Lead (see Key Personnel and Contact Details above) for further advice about SNP array karyotyping, its role in the diagnostic process and interpretation alongside other diagnostic information.

References:

Primdahl H, Wikman FP, von der Maase H, Zhou XG, Wolf H, Orntoft TF. Allelic imbalances in human bladder cancer: genome-wide detection with high-density single-nucleotide polymorphism arrays. J Natl Cancer Inst. 2002 Feb 6;94(3):216-23.

Mohamedali, A., et al. (2007). "Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes." Blood 110(9): 3365-3373.

Mohamedali, A. M., et al. (2009). "Novel TET2 mutations associated with UPD4q24 in myelodysplastic syndrome." J Clin Oncol 27(24): 4002-4006.

Tiu, R. V., et al. (2011). "Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies." Blood 117(17): 4552-4560.

Jerez, A., et al. (2012). "Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis." Blood 119(25): 6109-6117.

Kulasekararaj, A. G., et al. (2013). "Recent advances in understanding the molecular pathogenesis of myelodysplastic syndromes." Br J Haematol 162(5): 587-605.

Mohamedali, A. M., et al. (2013). "Utility of peripheral blood for cytogenetic and mutation analysis in myelodysplastic syndrome." Blood 122(4): 567-570.

Mohamedali, A. M., et al. (2015). "High concordance of genomic and cytogenetic aberrations between peripheral blood and bone marrow in myelodysplastic syndrome (MDS)." Leukemia 29(9): 1928-1938.

7.11 Translocation assays

7.11.1 BCR-ABL t(9;22)

Chronic Myeloid Leukaemia (CML) accounts for 15-20% of adult leukaemias. CML is a clonal haematopoietic stem cell malignancy that results in the formation of a BCR-ABL cytoplasmic fusion oncoprotein as the result of the reciprocal translocation between the long arms of chromosome 9 and chromosome 22 t(9;22) The disease is characterized by excess proliferation of myeloid progenitors that retain the capacity for differentiation during the stable or chronic phase of the disease. In addition, the BCR-ABL fusion is also seen in ALL albeit more rarely. In this disease the BCR-ABL fusion is the result of a slightly different break point and results in the p190 BCR-ABL fusion protein.

In addition, several rare BCR–ABL variant fusion genes have been described, such as the variants that result in the p195, p200, p225 and P230 BCR–ABL fusion proteins. We utilise a multiplex PCR which includes primer combinations that are able to co-amplify 14 different BCR-ABL1 fusion transcripts (P210 transcripts e13a2, e13a3, e14a2 & e14a3; P190 transcripts e1a2 & e1a3; P230 transcripts e19a2 & e19a3; P195 transcripts e6a2 & e6a3; P200 transcripts e8a2 & e8a3; P225 transcripts e18a2 & e18a3) and the internal loading control (BCR) in a single tube. The fragments generated by PCR will be run by capillary electrophoresis on the 3130 XL analyser and sized using a size standard to determine the exact length of the fragments generated, thus determining the specific BCR-ABL1 transcript.

Once the BCR-ABL1 transcript is identified a quantitative PCR (Q-PCR) utilizing the Applied Biosystems StepOne Plus platform is used for the highly sensitive quantitative detection of BCR-ABL fusion transcripts (p210 and p190) in order to monitor disease progression and residual disease. The procedure used at KCH is harmonized to the Europe Against Cancer (EAC) guidelines to ensure that we provide highly accurate and comparable BCR-ABL / ABL transcript ratios. In addition, we are one of a small group of laboratories in the UK currently engaged with the regional genetics laboratory to obtain an international conversion factor. This will enable our results to be directly compared with those of any other participating laboratory across the world. This provides even greater consistency which is desirable particularly in multi centre drug trials. Our conversion factor exercise will be completed in early 2010. A separate Q-PCR assay is used to detect the p190 variant. The rarer BCR-ABL1 variants are forwarded on to laboratories who quantitatively monitor those transcripts.

Persistent Q-PCR positivity after treatment is indicative of possible drug resistance and predictive of clinical relapse. A molecularly negative result is associated with disease free survival. Patients who convert to Q-PCR positivity after a negative result are at high risk of relapse. These patients may be candidates for 2nd and 3rd generation tyrosine kinase inhibitors. Failure to respond optimally to Imatinib can be caused by mutation in the p-loop domain of the translocated ABL kinase gene. Our repertoire of tests within the LMH also includes *abl* kinase p-loop mutation screening which can be carried out on samples previously sent to the laboratory for BCR-ABL monitoring by request

7.11.2 RUNXI-RUNXTI t(8;21)

The t(8;21) translocation is detected in approximately 7% of de novo AML (20-40% in the M2 subtype). The fusion gene encodes a chimeric protein, which blocks myeloid differentiation and may be important in myeloid leukaemogenesis. AML patients with t(8;21) have a relatively good prognosis.

This PCR reaction is designed to detect the fusion transcript AML1-MTG8(ETO) in t(8:21) positive AML patients at presentation and for accurate monitoring of residual disease level to identify patients ate high risk of relapse.

References: Tobal K, Yin JA. Monitoring of minimal residual disease by quantitative reverse transcriptase-polymerase chain reaction for AML1-MTG8 transcripts in AML-M2 with t(8; 21). Blood. 1996 Nov 15;88(10):3704-9.

Tobal K., et al (2000) Molecular quantitation of minimal residual disease in acute myeloid leukemia with t(8;21) can identify patients in durable remission and predict clinical relapse. Blood. 2000 Feb 1;95(3):815-9.

Van Donegan J.J et al 1999 Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease in acute leukemia. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 13 1901-1928

7.11.3 PML-RARA; t(15;17)

This PCR reaction is designed to detect the fusion transcript PMLRARA in patients with APML at presentation and for monitoring the disease.

The t(15;17) chromosomal translocation is a specific characteristic associated with acute promyelocytic leukaemia (APL, AML –M3). Two chimeric genes PML-RARA and RARA-PML are detected in 100% and 40% of AML patients respectively. The PML breakpoints cluster in three regions intron 6 BCR-1 (L-long-form), exon 6 BCR-2 (V-variable-form) or intron 3 BCR-3 (S-short-form) that fuse with the 3 prime portion of RARA intron 2. The isoforms occur in 55-60%, 35-40% and 8% of APL patients respectively. Several groups have reported that molecular monitoring of PML-RARA to evaluate MRD is clinically valuable in APL.

RT-PCR positivity after treatment is predictive of clinical relapse and a negative result is associated with disease free survival. Patients who convert to PCR positivity after a negative result are at high risk of relapse.

References:

Tobal et al (2001). Monitoring minimal residual disease and predicting relapse in APL by quantitating PML-RAR alpha transcripts with a sensitive competitive RT-PCR method. Leukemia. 15(7):1060-5.

Lo Coco F et al 1992 Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukemia. Lancet 340 1437-1438

Gallagher RE et al. 2003. Quantitative real-time RT-PCR analysis of PML-RAR alpha mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood;101(7):2521-8.

Grimwade 2002. The significance of minimal residual disease in patients with t(15;17).

Best Pract Res Clin Haematol;15(1):137-58. Review.

7.11.4 CBFB-MYH11 INV(16)

This PCR reaction is designed to detect this fusion transcript in patients with AML at presentation and for monitoring the disease.

The CBFB gene encodes a Beta subunit of translocation factor, CBFB, and alterations of CBFB are closely associated with AML. The MYH11 gene encodes the smooth muscle myosin heavy chain. The chimeric gene contains the 5 prime region of CBFB gene fused to the major region of MYH11 gene. The fusion protein impairs neutrophil development and alterations in the CBFB gene can contribute to the genesis of myelodysplasia.

The inversion of chromosome 16 (inv 16) and the translocation t(16;16) is a characteristic chromosomal aberration found in the M4Eo subtype of acute myeloid leukaemia (AML). This abnormality is detected in 7-10% of de novo AML (90% of M4Eo subtype) but is not limited to this subtype. The alternative splicing of the genes result in different transcripts. The majority of individuals with AML have type A, type D and E is found in a minority.

References: Shurtleff S.A. et al 1995 Heterogeneity in CBF beta/MYH11 fusion messages encoded by the inv(16)(p13;22) and the t(16;16)(p13;22) in acute myelogenous leukemia. Blood 85 3695-3703

Liu P et al 1993 Fusion between transcription factor CBF beat/PEBP2 beta and myosin heavy chain in acute myeloid leukemia. Science 261 1041-1044

Tobal et al 1995. Detection of CBFB/MYH11 transcripts in patients with inversion and other abnormalities of chromosome 16 at presentation and remission. Br J Haematol;91(1):104-8.

Van Donegan J.J. et al 1999 standardised RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease in acute leukemia. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 13 1901-1928

Kogan S.C. et al 1998 The PEBP2-beta-MYH11 fusion created by inv(16)(p13:22) in myeloid leukemia impairs neutrophil maturation and contributes to granulocytic dysplasia. Proc. Natl. Acad. Sci 95 11863-1186

Factors affecting results or interpretation of translocation assays: Translocation assays are performed from RNA which is very susceptible to degradation. Fresh samples give the best results and are more likely to pass our quality control. Samples received in heparin are not amenable to PCR based analysis and can therefore not be tested for BCR-ABL1 translocations.