**Title: KHMDC Oncology Cytogenetics User Guide**
**Subject: Investigations performed in KHMDC Cytogenetics**

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# KHMDC Oncology Cytogenetics User Guide

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# 1. Introduction

Viapath is a unique partnership of clinical, scientific and operational expertise, with a mission to transform pathology services in the UK. Our organisation is built on scientific expertise, providing a service that helps clinicians create better outcomes for their patients every day.

Our full-service, customer-focused offer is strongly rooted in the patient pathway. We serve our founding NHS Trusts, other NHS and private hospitals, and the GP community at large.

We are continually focused on innovation, finding new and improved ways to manage the logistics of high-volume pathology testing as well as specialist reference testing. We always strive to improve capabilities to better meet our customers' needs.

The HMDC at King's College Hospital is a regional centre for diagnostic services, providing Immunophenotyping, Cytogenetic, Molecular Diagnostic and Histopathology services covering most of South-East England.

The Cytogenetics laboratory at KHMDC offers an extensive testing repertoire, to aid the accurate diagnosis and prognosis of bone marrow disorders, currently utilising on-screen G-banded chromosome analysis, both manual and automated FISH analysis, and the provision of SNP array interpretation.

The Viapath Cytogenetics laboratory at KHMDC is a UKAS accredited medical laboratory, no. 9092.

# 2. Contact Details

**Correspondence Address:**
Viapath Cytogenetics (KHMDC)
Ground Floor, Hambleden Wing
King's College Hospital
Denmark Hill
London SE5 9RS

**Sample Address**:
King's HMDC Laboratory
c/o Central Specimen Reception
Blood Sciences Laboratory
Ground Floor, Bessemer Wing
King's College Hospital
Denmark Hill
London SE5 9RS

General Enquiries:
Email: kch-tr.cytogeneticslaboratory@nhs.net
Phone: 0203 299 7636/7

# 3. Main Departmental Contacts

**Head of Laboratory**

Robert Dunn DipRCPath robert.dunn@nhs.net

Tel 0203 299 7636

**Operations Leads/Principal Clinical Scientists**

Remi Oke remi.oke1@nhs.net

Zoë Thorn zoe.thorn@nhs.net

**Laboratory Administrative Assistant**

Nyakeh Richards nyakeh.richards@nhs.net

# 4. Hours of Operation

**Monday to Friday** 9.00am to 5.30pm

Weekends: There is no routine service at weekends. Samples requiring special attention should be arranged in advance.

Bank Holidays: The department is not staffed on Bank Holidays. An email is sent to regular customers in advance detailing arrangements at Christmas and Easter.

# 5. Sample Types

Bone Marrow is the tissue of choice to investigate patients suspected of having leukaemia or related haematological neoplasms. Peripheral Blood can be sent if disease cells are present in sufficient numbers to allow cell culture. This is satisfactory for CLL if there is peripheral blood lymphocytosis.

**N.B. Bone marrow should be sent in transport medium provided by the Cytogenetics department or in lithium/sodium heparin.**

The laboratory will provide media tubes to regular referrers for bone marrow. These tubes contain heparinised tissue culture medium to facilitate the transport of small volumes of bone marrow.

An allocation of transport media tubes will be issued every two weeks based on the number of samples usually returned to the laboratory in the specialised media. Sample volume data is reviewed annually, however, additional tubes can be sent upon telephone or email request by post, where necessary.

A **lithium heparin or sodium heparin** vacutainer can be used if transport medium is unavailable.

**N. B. Please DO NOT use other anticoagulants such as EDTA, which is toxic to cells, if G-banded chromosome analysis is requested. EDTA is acceptable for FISH only requests. For myeloid referrals please send both a lithium/sodium heparin sample and an EDTA.**

# 6. Dispatch of Samples

To provide an accurate result, samples for the laboratory must be sent in accordance with guidelines to ensure they arrive in a fit condition to be analysed.

World Health Organisation Guidance (2005) states that: "Shippers of infectious substances must ensure that packages are prepared in such a manner that they arrive at their destination in good condition and present no hazard to others during transport."

Similarly, under various dangerous goods transport/carriage regulations (see below *), it is the responsibility of the consignor (sender/requester) to ensure that all dangerous goods, including diagnostic specimens, are correctly classified and packaged into suitable containers that are correctly marked and labelled.

* The various Carriage Regulations consist of the European Agreement concerning the International Carriage of Dangerous Goods by Road 2015 (ADR 2015), The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations 2009 and The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment (Amendment) Regulations 2009, as amended 2011.

It is therefore the responsibility of the requestor to ensure that all samples are sent to Viapath in accordance with the following instructions.

## 6.1 Packaging requirements

Potentially infectious samples from GPs transported by designated vehicles provided by Viapath or the local NHS Trust must be carried out in compliance with the UK and European road transport regulations*.

Infectious substances include material that is known to contain, or is reasonably expected to contain, pathogens. When in transport, infectious substances must be packaged according to the UN Packing Instruction 650 as follows:

- All samples in containers (e.g. tube, pot known as the "primary") must be placed in individual plastic 'kangaroo' type sample bags to avoid cross contamination. **Never send samples from different patients in the same 'kangaroo' sample bag.** Where the primary contains a liquid, then the primary container must be leak proof. Where the primary contains

a solid, then the primary container must be sift proof (impermeable to dry contents).

- Individual sample bags should be placed into large, clear, sealable, leak proof, plastic, sample bags (known as the "secondary") that, where the specimen is a liquid, contains absorbent material sufficient to absorb the entire quantity of the liquid present in the specimen container (e.g. a sufficient amount of paper towelling to absorb any leakage).
- The referral paperwork should be contained in the secondary packaging pocket.
- The large bag should be placed into a suitable rigid sample transport container that meets the testing requirements of the regulations and is correctly marked and labelled.
- Only rigid outer containers supplied by Viapath or the local NHS Trust may be used to transport samples to the laboratory by road.
- There should be sufficient cushioning lining the outer rigid container to prevent samples becoming unstable.

**N.B. Please send samples at the earliest opportunity; samples must be received within 48 hours to ensure sample viability. Samples not sent immediately should be refrigerated at 4ºC and sent at the earliest opportunity.**

**It is advisable to telephone regarding samples that could arrive at the laboratory late in the day or out of hours. A Clinical Scientist may advise sending the sample the following day.**

**All Friday samples should arrive before 3pm to allow time for culture over the weekend. Myeloma samples need to arrive before 10am on a Friday to allow for sample separation.**

## 6.2 Request/Referral Forms

Please use the King's HMDC Referral Form [LF-IOG-REF v16] which may be retrieved from:
http://www.viapath.co.uk/departments-and-laboratories/hmdc-cytogenetics-laboratory-at-kings

The reason for referral is important to determine which culture types need to be set up, which tests to perform, numbers of cells to analyse and sample prioritisation. All relevant clinical and haematological information and likely diagnosis can be included. If the patient is a participant of a research trial, it is important to give details as certain trials can have specific analysis requirements.

The department operates a specimen acceptance policy. The following details are essential requirements for request cards. Samples referred without at least three patient identifiers may not be processed.

**Request forms must contain the following information:**

- Patient's forename and surname
- Patient's date of birth
- Patient's genetic sex

- Requestor's name and location:
  - Internal Request - location (ward code) and clinician details/code
  - External Request - address label/surgery and Consultant details.
- NHS and Hospital number
- Type of specimen(s)
- Date & time of specimen collection
- High risk for bacterial or viral infection or confirmed high risk infection; High risk specimens must be identified to the laboratory using the referral form (*Please note: without this information the specimen **will not** be processed by the laboratory*).
- Test(s) required
- Relevant clinical information, patient history and any transplant donor sex
- Request forms must be dated and signed by those taking the specimen. Please include appropriate contact details. (*Please note: without this information the specimen **will not** be reported by the laboratory*).

## 6.3 Rejection of Unacceptable Specimens

Specimens and request forms are checked on receipt to confirm the patient identification (PID) information provided on the form and specimen agree. A minimum of three PID data items (e.g. surname, forename, DOB and/or hospital number) are required by the laboratory and must match for the specimen to be accepted. Please ensure PIDs and contact details are ***clear and legible*** on all referral forms sent to KHMDC.

Samples without any patient identifiers are discarded and **not processed**.

## 6.4 Policy for High Risk Samples

All samples from patients at high risk of infection referred for cytogenetic analysis **must** be identified to the laboratory.

**The sample and request form must be clearly labelled as High Risk.**

*Please note: Specimens indicated as high risk without identification of the pathogen(s) will not be processed by the laboratory and communication with the referring provider will be attempted. If no response after 48 hours the sample will be disposed of.*

**HIGH RISK DISEASES**

Anthrax
Brucellosis
Creutzfeldt-Jakob Disease
Ebola
E. coli 0157 Infection
Hepatitis B
Hepatitis C

HIV
Severe Acute Respiratory Syndrome (SARS)
TB
Typhoid or Paratyphoid fever
Viral haemorrhagic fever (VHF) of any type

**Please note any ACDP (Advisory Committee on Dangerous Pathogens) category 3 pathogen (such as TB) or higher will not be processed by the laboratory as it does not have the sufficient containment level.**
**The Health & Safety Executive's approved list of biological agents can be found on their website:**
http://www.hse.gov.uk/pubns/misc208.pdf

# 7. Reporting

## 7.1 Results-online

Kings College Hospital laboratories offer test results online for NHS healthcare professionals. This is a free, secure, electronic, pathology results on-line service and is available to registered users. Please contact Viapath Customer Support on 0203 299 3576 if you would like to register for access to this service as a new user at an existing referral site.

## 7.2 Policy for Faxing Reports

KHMDC Cytogenetics does not issue reports by fax.

## 7.3 Posting / E-mailing Reports

Full copies of authorised reports are sent by post to external referrers, and can be emailed as PDFs using a group nhs.net account. Email is the preferred medium to enable quick reporting; please contact the laboratory to enable this service, and stop paper reports if no longer required. Results may also be requested by telephone in cases of urgent samples.

## 7.4 Additional Testing

Requests for additional tests on a specimen referral can be made by telephone or email if clinically relevant and agreed with a HCPC registered Clinical Scientist. This is subject to sufficient sample material availability.

## 7.5 Samples requiring further information

All samples that are not urgent and have an uncertain diagnosis will be held pending further information. Samples referred with urgent referral indications will be processed as appropriate for the disease until additional information is received that indicates tests should be cancelled. Further details are obtained from testing performed by other labs within KHMDC, or by telephone or email to the consultant listed on the referral form. This

information will be used to decide on the clinical validity of processing the sample; certain samples may have no cytogenetic testing performed, and may be referred for more relevant testing where indicated.

**N.B. Consultants are requested to co-operate as fully as possible with this policy; please respond to requests for further clinical information within 7 days otherwise samples will not be analysed. This is to avoid unnecessary work and helps the laboratory to process its large workload.**

### 7.6 Reporting Times

| Disease Type | Reporting Time (calendar days unless stated ) | Priority |
|---|---|---|
| Presentation ALL | Preliminary FISH = 3 working days<br>Full analysis = 14 | Urgent |
| Presentation AML | APML FISH = 3 working days<br>Full analysis = 14 | Urgent |
| Presentation CML | Preliminary FISH = 3 working days<br>Full analysis = 14 | Urgent |
| Relapse ALL/AML/CML samples | 14 | Urgent |
| MPN | 21 | Routine |
| MDS | 21 | Routine |
| Myeloma | 21 | Routine |
| CLL | 21 | Routine |
| Lymphoma | 21 | Routine |
| Other haematological | 21 | Routine |
| All follow-up samples | 21 | Routine |
| **The Association of Clinical Genomic Science (ACGS) Guidelines state that 95% of the samples should be reported within the guideline time (calendar days unless otherwise stated). All reporting times are subject to change during periods of insufficient staffing levels.** | | |

## 8. Laboratory Storage of samples

All samples for cytogenetic testing are stored in accordance with the guidelines issued by the Royal College of Pathologists in April 2015[8].

Bone marrow and peripheral blood samples referred to KHMDC Cytogenetics are disposed 48 hours after authorisation of the report. Cytogenetic preparations (stained slides) are kept for two years after the final report. Digitised images are stored with maintained accessibility for a minimum of 30 years. Fixed cytogenetic cell suspensions are stored for 6 months from receipt of sample. Fluorescence *In-Situ* Hybridisation (FISH) slides are disposed 48 hours after the final written report has been authorised. A representative photographed or digitised image is captured for all patients and stored with maintained accessibility for a minimum of 30 years.

# 9. Techniques

## 9.1 Chromosome analysis

Chromosome analysis is the microscopic examination of chromosomes in dividing cells. Such analysis can detect changes in chromosomal number and structure. Neoplasia may result from acquired cytogenetic abnormalities in otherwise normal individuals. Chromosome analysis allows a whole genome screen at a resolution of 3-10Mb. Tissue needs to be as fresh as possible with viable disease cells present. Cells are processed and stained using banding techniques to produce a karyotype. Abnormalities are defined and described according to the International System for Human Cytogenomic Nomenclature (ISCN) 2016.

**N.B. Analysis may not detect subtle chromosomal abnormalities or clones not represented in dividing cultured cells.**

## 9.2 Fluorescence *In-Situ* Hybridisation (FISH)

FISH is based on DNA probes annealing to specific target sequence of specimen DNA. Attached to the probes are fluorescent molecules which confirm the presence or absence of a particular genetic aberration when viewed under fluorescence microscopy.

# 10. Summary of Services Offered for Routine Cytogenetics and FISH

*Additional testing required outside of KHMDC Cytogenetics testing algorithms may be requested if the patient is a participant of a research trial, however, these requests should be clearly indicated on the referral form.*

## 10.1 Myeloproliferative neoplasms

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | CML indicated:<br>• FISH for *BCR-ABL1* [t(9;22)(q34;q11.2)]<br>• Full karyotype on bone marrow aspirate<br><br>MPN*[1]<br>• Single nucleotide polymorphism array (SNP-A)*[2] performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br><br>*[1] Where testing for mutation of *JAK2* | 3 working days<br><br>14 calendar days<br><br>15 working days |

| | | |
|---|---|---|
| | V617F or Exon 12, *CALR* and *MPL* has already been performed and with indication that the patient is fit for intensive treatment or transplant eligible.<br><br>*[2] This test will detect regions of chromosome imbalance at higher resolution than G-banded analysis where present in ≥10% cell population and regions of CN-LOH, but will not detect balanced rearrangements or the presence of independent clones. | |
| Monitoring | CML:<br>• Monitoring karyotype at 3, 6 and 12 months until CCyR achieved on bone marrow aspirate*.<br><br>* Where appropriate patients should be monitored using a molecular genetic test to detect gene fusion transcripts instead of cytogenetic methods. Detection of *BCR-ABL1* [t(9;22)] is offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC[‡].<br><br>• Full karyotype at treatment failure, progression or where Ph negative abnormal clones present.<br><br>MPN:<br>• Monitoring SNP-A/FISH as indicated to detect diagnostic chromosomal abnormality | 21 calendar days<br><br>5 working days<br><br>21 calendar days<br><br>21 calendar days |
| Transformation | • Per diagnostic work-up according to disease present | According to disease present |

[‡]This test utilises rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations" for further information available here: http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

## 10.2 Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | • FISH for *PDGFRA* (4q12), *PDGFRB* | 21 calendar days |

| | | |
|---|---|---|
| | (5q32), *FGFR1* (8p11) & *JAK2* (9p24) rearrangements | |
| Monitoring | • Detection of *PDGFRA* (4q12) is offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC‡. | 20 working days |

‡This test utilises rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations" for further information available here: http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

## 10.3 Myelodysplastic/myeloproliferative neoplasms

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | • FISH/PCR for *BCR-ABL1* [t(9;22)(q34;q11.2)] in cases of CMML<br>• Single nucleotide polymorphism array (SNP-A)* performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br><br>* This test will detect regions of chromosome imbalance at higher resolution than G-banded analysis where present in ≥10% cell population and regions of CN-LOH, but will not detect balanced rearrangements or the presence of independent clones. | 21 calendar days<br><br>15 working days |
| Monitoring | • Monitoring SNP-A/FISH as indicated to detect diagnostic chromosomal abnormality | 21 calendar days |
| Transformation | • Per diagnostic work-up according to disease present | According to disease present |

## 10.4 Myelodysplastic syndromes

**Please note: referring clinicians must provide the Cytogenetics laboratory with morphology and/or immunophenotyping studies [if not requested to be performed within KHMDC]; failure to provide this information will result in the sample being delayed. Fixed cells are stored for 6 months and testing may be requested at a later point upon receipt of this clinical information. Referrers are**

**encouraged to provide an appropriate email address for this communication.**

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | High risk MDS (MDS with excess blasts [MDS-EB], therapy-related [tMDS], or evidence of transformation):<br>• Full karyotype (+ FISH as indicated)<br>• Those with a failed karyotype will have a FISH panel performed to detect:<br>○ inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*,*MECOM*<br>○ t(6;9)(p23;q34.1); *DEK-NUP214*<br>○ t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*<br>○ t(?;21); *RUNX1* rearrangement<br>○ t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*, and *KMT2A* variants<br>○ inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*<br><br>A SNP-A*[1] will also be performed where possible; where not possible FISH for chromosomes 5 and 7 and *TP53* will also be performed<br><br>Low risk MDS or Aplastic Anaemia:<br>• Single nucleotide polymorphism array (SNP-A)*[2] performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br><br>*[1] This test will detect regions of chromosome imbalance at higher resolution than G-banded analysis where present in ≥10% cell population and regions of CN-LOH, but will not detect balanced rearrangements or the presence of independent clones. To generate an IPSS-R score, a G-banded karyotype can be requested. | 21 calendar days<br><br>SNP-A: 15 working days |
| Monitoring | • Monitoring SNP-A/karyotype/FISH as indicated to detect diagnostic chromosomal abnormality | 21 calendar days |
| Progression to MDS-EB/ AML | • Per diagnostic work-up according to disease present | According to disease present |

## 10.5 Acute myeloid leukaemia

Standard investigations:

| Stage | Investigations | Turnaround Time |
|---|---|---|
| Diagnosis | • Full karyotype (with exception of cases positive for *PML-RARA*; *RUNX1-RUNX1T1* or *CBFB-MYH11* for which a karyotype will not be performed unless specifically requested) | 14 calendar days |
| | • FISH for cryptic *KMT2A* (11q23) & *CBFB-MYH11* [inv(16) & t(16;16)] rearrangements, and *RARA* (17q21) rearrangement | FISH reported in advance of G-banding if clinically significant result |
| | • Patients eligible for treatment with Gemtuzumab Ozogamicin (Mylotarg®)[11] or CPX-351 (Vyxeos®)[12], or those with a failed karyotype will have a FISH panel performed to detect:<br>○ inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*,*MECOM*<br>○ -5/del5q<br>○ t(6;9)(p23;q34.1); *DEK-NUP214*<br>○ -7/del7q<br>○ t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*<br>○ t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*, and *KMT2A* variants<br>○ t(9;22); *BCR-ABL1*<br>○ inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*<br>○ del(17p); *TP53* deletion<br>○ t(?;17)(?;q21); *RARA* rearrangement | For urgent treatment:<br>4 calendar days<br><br>Failed karyotype:<br>14 calendar days |
| | • FISH for *PML-RARA* [t(15;17)] for APL & FISH for *RARA* for variant APL to non-specifically detect:<br>○ t(5;17)(q35;q21); *NPM1-RARA*<br>○ t(11;17)(q23;q21); *ZBTB16* (*PLZF*)-*RARA*<br>○ t(11;17)(q13;q21); *NUMA1-RARA*<br>○ t(17;17)(q21;q21); *STAT5B-RARA* | 3 working days (target <24 hours) |
| Monitoring | • Monitoring karyotype/FISH* as indicated to detect diagnostic chromosomal abnormality | 21 calendar days |
| | * Where appropriate patients should be | 7 working days |

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|---|---|---|
| | monitored using a molecular genetic test to detect gene fusion transcripts instead of cytogenetic methods. Currently offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC are:<br>○ t(15;17); *PML-RARA*‡<br>○ t(8;21); *RUNX1-RUNX1T1*‡<br>○ inv(16) or t(16;16); *CBFB-MYH11*‡<br>○ t(9;22); *BCR-ABL1*‡ | |
| Relapse | • Full karyotype<br>• FISH as indicated to detect diagnostic abnormality/additional abnormalities<br><br>*If no previous cytogenomic testing, case will be treated as per diagnosis | 14 calendar days |

‡These tests utilise rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations" for further information available here:
http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

## 10.6 Acute leukaemias of ambiguous lineage

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | • Full karyotype<br>• FISH for *BCR-ABL1* [t(9;22)(q34;q11.2)] & *KMT2A* (11q23) | 14 calendar days<br>3 working days |
| Monitoring | • Monitoring karyotype/FISH* as indicated to detect diagnostic chromosomal abnormality<br><br>* Where appropriate patients should be monitored using a molecular genetic test to detect gene fusion transcripts instead of cytogenetic methods. Detection of *BCR-ABL1* [t(9;22)] is offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC‡. | 21 calendar days<br><br>5 working days |
| Relapse | • Full karyotype<br>• FISH as indicated to detect diagnostic abnormality/additional abnormalities<br><br>*If no previous cytogenomic testing, case will be treated as per diagnosis | 14 calendar days |

‡This test utilises rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations" for further information available here:
http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

## 10.7 Precursor lymphoid neoplasms

### 10.7.1 B-ALL

Standard investigations:

| Stage | Investigations | Turnaround Time |
|---|---|---|
| Diagnosis | Patients >40 years:<br>• FISH for *BCR-ABL1* [t(9;22)(q34;q11.2)] & *KMT2A* (11q23)<br>  ○ If negative: additional FISH testing for ABL-class fusions:<br>  ▪ *CRLF2* (Xp22.33,Yp11.2)<br>  ▪ *ABL1* (9q34)<br>  ▪ *ABL2* (1q25.2)<br>  ▪ *PDGFRB*,*CSF1R* (5q32)<br>• Karyotype to detect fusion partner if *KMT2A*, *ABL1*, *ABL2* or *PDGFRB*,*CSF1R* rearranged; *IGH* FISH followed by karyotype if *CRLF2* rearranged<br>• Single nucleotide polymorphism array (SNP-A)* performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br><br>Patients ≤40 years:<br>• FISH for *ETV6-RUNX1* [t(12;21)(p13;q22)], *BCR-ABL1* [t(9;22)(q34;q11.2)], *KMT2A* (11q23) & *TCF3* (19p13)<br>  ○ If negative: additional FISH testing for ABL-class fusions:<br>  ▪ *CRLF2* (Xp22.33,Yp11.2)<br>  ▪ *ABL1* (9q34)<br>  ▪ *ABL2* (1q25.2)<br>  ▪ *PDGFRB*,*CSF1R* (5q32)<br>• Karyotype to detect fusion partner if *KMT2A*, *ABL1*, *ABL2* or *PDGFRB*,*CSF1R* rearranged; *IGH* FISH followed by karyotype if *CRLF2* rearranged<br>• Single nucleotide polymorphism array (SNP-A)* performed by the Laboratory for Molecular Haemato- | 3 working days<br><br>14 calendar days<br><br><br><br><br><br>14 calendar days<br><br>15 working days<br><br><br><br>3 working days<br><br>14 calendar days<br><br><br><br><br><br>14 calendar days<br><br>15 working days |

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|---|---|---|
| | Oncology (LMH) in KHMDC<br><br>* This test will detect regions of chromosome imbalance at higher resolution than G-banded analysis where present in ≥10% cell population and regions of CN-LOH, but will not detect balanced rearrangements or the presence of independent clones. | |
| Monitoring | • Monitoring SNP-A/FISH* as indicated to detect diagnostic chromosomal abnormality<br><br>* Where appropriate patients should be monitored using a molecular genetic test to detect gene fusion transcripts instead of cytogenetic methods. Detection of *BCR-ABL1* [t(9;22)] is offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC‡. | 15 working days / 21 calendar days<br><br>5 working days |
| Relapse | • Single nucleotide polymorphism array (SNP-A) performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br>• FISH as indicated to detect diagnostic abnormality/additional abnormalities<br><br>*If no previous cytogenomic testing, case will be treated as per diagnosis | 15 working days<br><br>14 calendar days |

‡This test utilises rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations" for further information available here: http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

### 10.7.2 T-ALL / T-PLL

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | T-ALL:<br>• FISH testing for ABL-class fusions:<br>  ○ *CRLF2* (Xp22.33,Yp11.2)<br>  ○ *ABL1* (9q34)<br>  ○ *ABL2* (1q25.2)<br>  ○ *PDGFRB*,*CSF1R* (5q32)<br>  ○ *PDGFRA* (4q12)<br>• Single nucleotide polymorphism array (SNP-A)* performed by the Laboratory for Molecular Haemato- | 14 calendar days<br><br>15 working days |

| | | |
|---|---|---|
| | Oncology (LMH) in KHMDC<br><br>Bi-lineage or advanced disease suspected:<br>• FISH for *KMT2A* (11q23)<br><br>T-PLL:<br>• FISH for *MYC* (8q24), *ATM* (11q22.3) & *TRA*/*TRD* (14q11.2)<br><br>* This test will detect regions of chromosome imbalance at higher resolution than G-banded analysis where present in ≥10% cell population and regions of CN-LOH, but will not detect balanced rearrangements or the presence of independent clones. | <br><br><br><br>14 calendar days<br><br>21 calendar days |
| Monitoring, Follow up | • Monitoring SNP-A/FISH* as indicated to detect diagnostic chromosomal abnormality<br><br>* Where appropriate patients should be monitored using a molecular genetic test to detect gene fusion transcripts instead of cytogenetic methods. Detection of *BCR-ABL1* [t(9;22)] is offered by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC[‡]. | 15 working days / 21 calendar days<br><br>5 working days |
| Relapse | • Single nucleotide polymorphism array (SNP-A) performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br>• FISH as indicated to detect diagnostic abnormality/additional abnormalities<br><br>*If no previous cytogenomic testing, case will be treated as per diagnosis | 15 working days<br><br>14 calendar days |

[‡]This test utilises rt qPCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH KHDMC Laboratory User's Handbook Section 8: Additional Information on Molecular Investigations for further information available here:
http://www.viapath.co.uk/departments-and-laboratories/haematological-malignancy-diagnostic-centre

## 10.8 Mature B-cell neoplasms

### 10.8.1 Chronic Lymphocytic Leukaemia (CLL)

Standard investigations:

| Stage | Investigations | Turnaround |
|---|---|---|

| | | **Time** |
|---|---|---|
| Diagnosis | • SNP-A* performed by the Laboratory for Molecular Haemato-Oncology (LMH) in KHMDC<br>• *TP53* mutation performed by LMH in KHMDC<br><br>* This whole genome screen will detect additional abnormalities to those detectable by the standard FISH panel where present in ≥10% cell population, and can detect the two different classes of 13q14 deletion. | 15 working days<br><br>30 working days |
| Monitoring, pre – treatment | • FISH for *TP53* (17p13) deletion<br>• *TP53* mutation performed by LMH in KHMDC | 21 calendar days<br>30 working days |
| ?Richter's transformat-ion | • FISH to detect:<br>o *MYC* (8q24.1) rearrangement<br>o *IGH-MYC* [t(8;14)(q24.1;q32)]<br>o *IGH-BCL2* [t(14;18)(q32;q21)]<br>o *BCL6* (3q27) rearrangement | 14 calendar days |

### 10.8.2 Multiple myeloma (MM)

**N.B. Cytogenetics is not performed on Monoclonal Gammopathy of Uncertain Significance (MGUS), monitoring samples or those samples containing <1% malignant plasma cells by flow-cytometry.**

**Please note: referring clinicians must provide the Cytogenetics laboratory with plasma cell percentage as determined by morphology and/or immunophenotyping studies [if not requested to be performed within KHMDC]; failure to provide this information will result in the sample being delayed. Fixed cells are stored for 6 months and testing may be requested at a later point upon receipt of this clinical information. Referrers are encouraged to provide an appropriate email address for this communication.**

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | • FISH* to detect:<br>o *TP53* (17p13) deletion<br>o *CDKN2C* (1p32) deletion<br>o *CKS1B* (1q21) gain<br>o *IGH* (14q32.3) rearrangement‡<br>o *MYC* (8q24.1) rearrangement<br><br>‡Where *IGH* (14q32.3) is rearranged, sequential reflex FISH* for:<br>o t(4;14)(p16.3;q32); *IGH-FGFR3*<br>o t(11;14)(q13;q32); *IGH-CCND1*<br>o t(14;16)(q32;q23); *IGH-MAF* | 21 calendar days |

| | | |
|---|---|---|
| | ○ t(14;20)(q32;q12); *IGH-MAFB*<br>○ t(6;14)(p21;q32); *IGH-CCND3*<br><br>* Where sufficient $CD138^+$ cells are present in the sample | |
| Relapse | • FISH* for:<br>○ *TP53* (17p13) deletion<br>○ *CDKN2C* (1p32) deletion<br>○ *CKS1B* (1q21) gain<br>○ Diagnostic *IGH* t(14;*v*) rearrangement when present<br><br>* Where sufficient $CD138^+$ cells are present in the sample | 21 calendar days |

### 10.8.3 Non-Hodgkin's Lymphoma (NHL)

FISH analysis for NHL is carried out on uncultured fixed cells from peripheral blood sample or bone marrow aspirate, or from bone marrow smears. Selection of appropriate FISH test(s) will be performed in conjunction with clinical information/request, morphology, flow cytometry, histopathology and immunohistochemistry, and once bone marrow infiltration has been confirmed.

Standard investigations:

| **Stage** | **Investigations** | **Turnaround Time** |
|---|---|---|
| Diagnosis | • FISH as appropriate to detect:<br>○ *IGH-CCND1* [t(11;14)(q13;q32)]<br>○ *MYC* (8q24.1) rearrangement<br>○ *IGH-MYC* [t(8;14)(q24.1;q32)]<br>○ *IGH-BCL2* [t(14;18)(q32;q21)]<br>○ *BCL6* (3q27) rearrangement | 21 calendar days |
| Monitoring | • FISH to detect diagnostic rearrangement as indicated. | 21 calendar days |

## 11. Complaints and Compliments

The department has procedures for logging compliments and complaints from service users. Please contact the Head of Service for further details if required.

## 12. References

1. Swerdlow, S. H. *et al*. (2017) *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: International Agency for Research on Cancer (IARC).

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5. ACGS Chronic Myeloid Leukaemia & Other Myeloproliferative Neoplasms (2011) v1.00
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8. The Royal College of Pathologists: The retention and storage of pathological records and specimens (5th edition). 2015
9. Rajkumar, S.V. *et al*. (2014). International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*., 15(12), e538-48.
10. Manola, K. (2013). Cytogenetic abnormalities in acute leukemia of ambiguous lineage: an overview. *BJH*, 163(1):24-39.
11. Lambert, J. *et al.* (2019). Gemtuzumab ozogamicin for de novo acute myeloid leukemia: final efficacy and safety updates from the open-label, phase III ALFA-0701 trial. *Haematologica*, 104(1):113-119.
12. Lancet, J. E. *et al.* (2018). CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia. *Journal of Clinical Oncology*, 36(26):2684-2692.