# KHMDC Oncology Cytogenetics User Guide

## 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 better 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 KHMDC at Kings College Hospital is a Regional Centre for diagnostic services, providing Immunophenotyping, Cytogenetic, Molecular Diagnostic and Histopathology services covering most of South-East England.

The Oncology Cytogenetics laboratory at KHMDC Kings provides an extensive karyotyping and FISH service for a range of haematological referrals with referral numbers currently >5000 samples per year.

## Contact Details

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General Enquiries: 0203 299 7636 or 0203 299 7637

## Main Departmental Contacts

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## Hours of Operation

**Monday to Friday** 8.30am to 5pm
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.

### 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 FISH studies in CLL if there is peripheral blood lymphocytosis.

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

The laboratory will provide containers to regular referrers for bone marrow. These bottles contain heparinised tissue culture medium with antibiotics, to facilitate the transport of the small amount of bone marrow and avoid desiccation.

An allocation of specimen bottles will be issued at the beginning of each month based on the number of samples usually received. More bottles can be sent upon telephone or email request by hospital transport or by post, where necessary.

A blood tube containing **lithium heparin** can be used if transport medium is unavailable.

**Please DO NOT use other anticoagulants such as EDTA, which is toxic to cells.**

### Dispatch of Samples

In order to provide and accurate and reliable 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.

## Packaging requirements

Potentially infectious samples from GPs transported by designated vehicles provided by Viapath or the local NHS Trust must be carried 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 being transported infectious substances must be packaged according to the UN standard P650 (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. 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 large bag should be placed into a suitable rigid sample transport container that is capable of meeting the testing requirements of the regulations and that 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.
- If the rigid outer container becomes contaminated it must be disinfected by wiping out with 1000 ppm chlorine solution or equivalent.

***Please send samples at the earliest opportunity ideally within 24 hours. Samples not being sent immediately should be refrigerated at 4 C and sent at the earlies opportunity***
***It is advisable to telephone about any 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 ideally arrive before 3pm to allow time for culture over the weekend. Myeloma samples need to arrive before 1pm to allow for cell separation.**

## Request/Referral Cards

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 requirements, such as levels of analysis by cytogenetics and/or FISH.
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 set-up.

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

- Patient's first and family name
- Patient's date of birth
- Patient's gender
- Requestor's name and location -Internal Request - location (ward code) and clinician details/code -External Request - address label/surgery and GP details.
- NHS and/or Hospital number
- Type of specimen
- Date/time of specimen collection
- Test(s) required
- Relevant clinical information
- Request forms should be dated and signed by those taking the specimen.

***Samples referred without at least three patient identifiers may not be set-up.***
***INCOMPLETE FORMS WHICH DO NOT SPECIFY CYTOGENETIC TESTING MAY NOT BE PROCESSED.***

## Policy for High Risk Samples

All samples from patients at High Risk of infection referred for cytogenetic analysis should be identified to the laboratory. The sample and request card must be clearly labelled as High Risk.

HIGH RISK
Anthrax
Brucellosis
Creutzfeldt-Jakob Disease
E. coli 0157 Infection
Hepatitis B
Hepatitis C HIV
Severe Acute Respiratory Syndrome (SARS)
Tuberculosis
Typhoid or Paratyphoid fever
Viral haemorrhagic fever (VHF) of any type

Samples at risk of infection with HIV Hepatitis B or Hepatitis C, may be processed by the laboratory. However, as the samples require special attention it would be advisable to contact the laboratory prior to dispatch
Please note any ACDP category 3 pathogen or higher will not be processed by the laboratory.

***High Risk samples should not be processed over a weekend and samples can only be accepted on a Friday in exceptional circumstances. Full cytogenetic analysis will only be considered in circumstances where a result will directly influence patient management.***

Uncultured specimens can be fixed for FISH only but a full cytogenetic result will not be possible.
HIV, Hepatitis B and Hepatitis C samples received without strong indication for cytogenetic analysis will be fixed for FISH only if clinically relevant. Consequently, a conventional cytogenetics result will not be possible.
In all instances a report will be issued to the referring consultant.

## 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 (Surname, Forename, Date of Birth and hospital number) are required by the laboratory and these must match for the specimen to be accepted. If errors are found, the KHMDC reception laboratory staff will contact the requestor, explain the problem and request clarification.

Usually the requestor will be given the opportunity to complete patient information on the specimen or request and sign a disclaimer. If the specimen is tested the report will state the nature of the problem as a comment. Alternatively, the requesting clinician will be asked to send a repeat sample.

## Sample Prioritisation and Reporting Times

Sample prioritisation and reporting performance targets of the professional standards of the Association of Clinical Cytogenetics are used as minimum standards, including General Best Practice Guidelines, Haemato-Oncology Best Practice Guidelines, Guidelines for FISH Scoring in Oncology and disease-specific Guidelines for CML/MPN, AML/MDS and ALL.

### Reporting Times

| Disease Type | Reporting Time (calendar days) | Priority |
|---|---|---|
| Urgent FISH | 3 | Urgent |
| ALL | Preliminary result = 3<br>Full analysis = 14 | Urgent |
| AML | 14 | Urgent |
| CML | Preliminary result =3<br>Full analysis = 14 | Urgent |
| Relapse samples | 14 | Urgent |
| MPD | 21 | Routine |
| MDS | 21 | Routine |
| Myeloma | 21 | Routine |
| CLL | 21 | Routine |
| Other haematological | 21 | Routine |
| Lymphoma | 21 | Routine |
| All follow up samples | 21 | Routine |
| **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.** | | |

### Samples requiring further information

All samples that are not urgent and have an uncertain diagnosis will be held pending further information. Further details are obtained on the internal IOG system, by telephone or email. This information will be used to make a decision on the clinical validity of processing the sample and may result in a sample being not required or referred for more relevant molecular testing

**Consultants are requested to cooperate as fully as possible with this policy. This is to avoid unnecessary work and helps the laboratory to process its large workload.**

### Telephone enquiries

Telephone enquiries are welcome. Cytogenetics' staff will be pleased to accept requests to process samples if required urgently to determine treatment or by specific appointments and will make every effort to make results available. However please note this may not always be possible especially at short notice.

## Reporting

### Hard-copy Reports

Hard-copy reports will be sent to external referring consultant by first-class mail. Reports can be addressed to other designated persons, if requested to do so in writing by the consultant.

### 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 immediately available to all users of our pathology service, who have access to the NHS net.

Many of our clients have been using this service for a number of years and find it highly valuable. The system can be set up to notify you of new results and offers search facilities to identify specific results.

Please Note: This service is not for GP Practices.

Customers with access to the NHS net can preview the site at https://nww.resultsonline.kingsch.nhs.uk using the following test log-in details:

**Username:** aguest
**Password:** password

Existing customers with NHS net access, wishing to use the Results On-line service should e-mail kch-tr.pathIT@nhs.net.

### Policy for Faxing Reports
KHMDC Cytogenetics do not issue reports by fax.

### E-mailing Reports
Full copies of authorised reports can be emailed as PDF's using an nhs.net account. Please contact the laboratory if this service is required.

### Additional Testing

Requests for additional tests on a 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 being available.

### 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 'The retention and storage of pathological records and specimens' (5th edition).**
Bone marrow and peripheral blood samples referred to KHMDC Cytogenetics are disposed of after one week following set-up and culture. Cytogenetic preparations (stained slides) are kept for two years after the final report. Digitised images are stored with maintained accessibility for 30 years. Cytogenetic cell suspensions in fixative are stored for 1 year. Fluorescence in-situ hybridisation (FISH) slides are kept at least until the final written report has been authorised and issued. A representative photographed or digitised image is captured for all patients and stored with maintained accessibility for 30 years.

### Complaints and Compliments
The department has procedures for logging compliments and complaints from service users. Please feel free to contact the head of service for further details if required.

## Techniques

### 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 3-5Mb.Tissue needs to be as fresh as possible with viable disease cells. 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)

## Analysis criteria

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. Ten cells will be analysed and ten examined according to standard procedures and best practice on all diagnostic referrals requiring cytogenetics. If a diagnostic case is abnormal ten cells will be analysed but if there are insufficient metaphases clonal abnormalities will be reported in fewer cells. Monitoring karyotypes: Five cells will be analysed and twenty-five scanned according to standard procedures and best practice. If the referral shows persistent disease then five cells will be analysed and fifteen scanned.

***Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which analysis may not detect.***

Please note that referrals that have been significantly delayed in transit are noted in the final report. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which analysis may not detect.

## Fluorescence in situ hybridisation (FISH)

FISH is based on DNA probes annealing to specific target sequence of sample DNA. Attached to the probes are fluorescent reporter molecules which under fluorescence microscopy confirm the presence or absence of a particular genetic aberration when viewed under fluorescence microscopy. 100 to 150 interphase cells will be analysed depending on the referral reason.

# Summary of Services Offered for Routine Cytogenetics and FISH

## ACUTE MYELOID LEUKAEMIA (AML) /MYELODYSPLASTIC (MDS) CYTOGENETICS

### AML

AML is a neoplastic disorder that results from a block in the differentiation of haematopoietic Progenitor cells along with uncontrolled proliferation. Cytogenetics is one of the most important prognostic factors and WHO classification recognised recurrent cytogenetic abnormities in its revision in 2008.

Full karyotype at diagnosis.

Rapid FISH for t(15;17) at diagnosis in APML

FISH at diagnosis to detect t(8;21) [RUNX1-RUNX1T1], inv(16) [CBFB], 11q23 rearrangements [MLL] depending on morphology or as requested

Failed cases will have relevant FISH studies dependant on clinical indications as well as FISH for 5, 7 and TP53 to elucidate complex karyotypes.

### MDS

Approximately 50% of confirmed, de novo MDS cases have cytogenetic abnormalities at diagnosis, which helps to confirm the presence of a clonal disorder and aids the distinction between MDS and reactive causes of dysplasia.

Presentation MDS samples where G-banded analysis has failed have interphase FISH analysis carried out using the **EGR1/D5S23,D5S721** and **D7S486/CEP7** probe sets. Cep 8 can also be requested.

All patients have conventional cytogenetics performed at diagnosis, supplemented by FISH tests as appropriate to identify favourable and unfavourable prognostic abnormalities. Cytogenetics can be used to monitor response to treatment and can confirm relapse or indicate disease progression.

WHO 2016 states that in MDS the abnormality must be demonstrated by

conventional karyotyping, not by fluorescence in situ hybridization (FISH) or sequencing technologies. Therefore if conventional karyotyping fails another sample will be requested even in the presence of FISH abnormalities.

The presence of +8, −Y, or del(20q) is not considered to be MDS-defining in the absence of diagnostic morphologic features of MDS. Del(5q) remains as the only cytogenetic or molecular genetic abnormality that defines a specific MDS subtype. Based on recent data showing no adverse effect of 1 chromosomal abnormality in addition to the del(5q) the entity MDS with isolated del(5q) may be diagnosed if there is 1 additional cytogenetic abnormality besides the del(5q), unless that abnormality is monosomy 7 or del(7q). Even though cytogenetic findings are not used to define other specific subtypes of MDS, they are strongly correlated with prognosis, as reflected in the 5 cytogenetic prognostic groups in the IPSS-R.

| FISH Probe | Cytogenetic Abnormality | Disease/FAB |
|---|---|---|
| **RUNX1-RUNX1T1** | **t(8;21)** | **AML M2** |
| **PML-RARα** | **t(15;17)** | **APL** |
| **RARα** | **t(17;?)** | **varAPL** |
| **CBFβ** | **inv(16)/t(16;16)** | **AML M2Eo/M4Eo** |
| **KMT2A (MLL)** | **11q23** | **AML M4/M5** |
| **BCR-ABL1** | **t(9;22)** | **AML** |
| **EGR1/D5S23,D5S721** | **del(5q31)/-5** | **AML/MDS/5q-syndrome** |
| **D7S486/CEP7** | **del(7q31)/-7** | **AML/MDS** |
| **D20S108** | **del(20q)** | **MDS** |
| **Chromosome 8 alpha satellite** | **Trisomy 8** | **AML/MDS** |
| **ETV6** | **t(12p13;?)** | **AML/MDS** |
| **MECOM (EVI1)** | **inv(3)/t(3;3)** | **AML/MDS** |
| **TP53** | **17p13** | **AML/MDS** |

# ACUTE LYMPHOBLASTIC LEUKEMIA INCLUDING PRECURSOR NEOPLASMS

Acute Lymphoblastic leukaemia is a neoplastic disorder that results in the accumulation of lymphocytes in the bone marrow. It can be subdivided according to the level of maturity of the lymphocytes.

All patients have conventional cytogenetics performed at diagnosis, supplemented by FISH tests as appropriate to identify favourable and unfavourable prognostic abnormalities. Cytogenetics can be used to monitor response to treatment and can confirm relapse or indicate disease progression.

BCR-ABL1 is the primary FISH test carried out on all ALL referrals where the patient is >26 years followed by KMT2A if BCR-ABL1 negative. ETV6-RUNX1 is the primary FISH test carried out on all ALL referrals where the patient is <26 years followed by BCR-ABL1,KMT2A and TCF3.

*WHO 2016 now includes B-ALL with translocations involving tyrosine kinases or cytokine receptors ("BCR-ABL1–like ALL"). The cases with translocations involving tyrosine kinase genes involve many different genes including* ABL1 *(with partners other than* BCR*), as well as other kinases including* ABL2*,* PDGFRB*,* NTRK3*,* TYK2*,* CSF1R*, and* JAK2*. Over 30 different partner genes have been described. Some patients, especially those with* EBF1-PDGFRB *translocations, have shown remarkable responses to TKI therapy, even after failing conventional therapy. Therefore FISH studies for JAK2 and PDGFRB will be included where morphology and/or other clinical features are suggestive.*

| FISH Probe | Cytogenetic Abnormality |
|---|---|
| **BCR-ABL1** | **t(9;22)** |
| **ETV6-RUNX1** | **t(12;21)** |

| | |
|---|---|
| **KMT2A (MLL)** | **11q23** |
| **TCF3(E2A)** | **t(1;19)/t(17;19)** |
| **ETV6** | **dic(9;12)** |
| **Enumeration of 4, 14, 17, 18, 21** | **Hyperdiploidy** |
| **PDGFRB** | **5q32** |
| **JAK2** | **9p13** |

## MDS/MPN

The MDS/MPN group is made up of chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (aCML), a 'provisional entity', refractory anemia with ring sideroblasts and thrombocytosis (RARS-T), and a 'by exclusion' subcategory, MDS/MPN unclassified (MDS/MPN-U)

Chronic Myelomonocytic Leukaemia (CMML) samples will always have **PDGFRβ** FISH carried out in the absence of a cytogenetically detectable t(5;12)(q33;p13) and eosinophilia is present.

All patients have conventional cytogenetics performed at diagnosis, supplemented by FISH tests as appropriate to identify favourable and unfavourable prognostic abnormalities. Cytogenetics can be used to monitor response to treatment and can confirm relapse or indicate disease progression.

| Probe | Cytogenetc Abnormality | Disease |
|---|---|---|
| **EGR1/D5S23,D5S721** | **del(5q31) /-5** | **AML/MDS/5q- syndrome** |
| **D7S486/CEP7** | **del(7q31)/ -7** | **AML** |
| **D20S108** | **del(20q)** | **MDS** |
| **Chromosome 8 alpha satellite** | **Trisomy 8** | **AML/MDS** |
| **Chromosome 9 alpha satellite** | **Trisomy 9** | **MDS/MPN** |
| **PDGFRβ** | **t(5q33)** | **CMML** |

## Myeloproliferative neoplasm (CML)

Chronic myeloid leukaemia is a myeloproliferative neoplasm of which the cytogenetic hallmark is the t(9; 22)(q34; q11) Philadelphia chromosome translocation that can be detected by chromosome analysis in 90-95% of cases at diagnosis. This translocation gives rise to a BCR/ABL1 gene fusion gene on the derived 22 (Ph chromosome). The remaining 5-10% of cases have variant translocations or cryptic rearrangement, the latter, which can be detected by FISH.

FISH is performed at diagnosis to detect BCR-ABL1 gene rearrangement on all cases for rapid confirmation of BCR-ABL1 status and to establish a signal pattern for future monitoring. FISH is also used to detect the BCR-ABL1 gene rearrangement in cases with normal cytogenetics and cryptic BCR-ABL1 in cases with variant translocations involving other translocations involving other chromosomes and in the small number of cases that fail to grow in culture.

Karyotyping is performed during monitoring whenever there is a change in bone marrow morphology or clinical response to treatment. During accelerated phase additional cytogenetic changes may occur in $Ph^+$ cells that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.

With regard to chronic myeloid leukemia (CML), $BCR\text{-}ABL1^+$, most cases of CML in chronic phase can be diagnosed from peripheral blood (PB) findings combined with detection of t(9;22)(q34.1;q11.2) or, more specifically, *BCR-ABL1* by molecular genetic techniques. However, a bone marrow (BM) aspirate is essential to ensure sufficient material for a complete karyotype and for morphologic evaluation to confirm the phase of disease (WHO 2016)

### BCR-ABL1 FISH is carried out on all diagnostic or potential CML cases irrespective of karyotype

| Probe | Abnormality | Sub-type of MPN |
|---|---|---|
| **BCR-ABL1** | **t(9;22)/var/cryptic /del der(9)** | **CML** |

## Myeloproliferative neoplasias (non - CML)

The BCR/ABL1 negative myeloproliferative neoplasms are a heterogeneous group of clonal stem cell disorders. They are represented in the WHO classification by a number of different disease categories: classic MPNS (PV, ET and PM), MPN overlapping with MDS and eosinophillic neoplasms. The distinction of MPN from CML requires exclusion of t(9;22)/BCR/ABL1. This test is offered where morphology is suggestive of a non-classic MPN.

The most common MPN associated with PDGFRA rearrangement is that associated with FIPILI-PDGFRA formed as a cryptic deletion of 4q12. Presentation is generally as CEL.A distinctive type of myeloid neoplasm occurs in association with rearrangement of PDGFRB at 5q31-33. Haematological malignancies with FGFR1 rearrangement at 8p11 can be found in both lymphoid and myeloid malignancy but presentation is most often CEL. In the WHO 2016 revision this disease group will incorporate the myeloid neoplasm with t(8;9)(p22;p24.1);*PCM1-JAK2* as a new provisional entity

| Probe | Abnormality | Sub-type of MPN |
|---|---|---|
| **4q12 tri-colour** | **4q12 translocation or cryptic 4q12 deletion** | **Hypereosinophilic Syndrome (HES)/Chronic Eosinophilic Leukaemia (CEL)** |
| **PDGFRβ** | **5q33** | **MDS/MPN** |
| **FGFR1** | **t(8p11;?)** | **8p11 Myeloproliferative syndrome** |
| **JAK2** | **t(8;9) *PCM1-JAK2*** | **MPN with eosinophilia** |

| Probe | Abnormality | Sub-type of MPN |
|---|---|---|
| **BCR-ABL1** | **t(9;22)/var/cryptic /del der(9)** | **Non classic MPN's** |

## T-cell Acute Lymphoblastic Leukaemia (T-ALL) and T-cell Prolymphocytic Leukaemia (T-PLL)

T-cell Acute Lymphoblastic Leukaemia (T-ALL), currently classified by the World Health Organization (WHO) as T lymphoblastic leukaemia/lymphoma, comprises
15% of paediatric and 25% of adult acute lymphoblastic leukaemia cases

All patients should have conventional cytogenetics performed at diagnosis, supplemented by FISH tests as appropriate to identify favourable and unfavourable prognostic abnormalities. Cytogenetics can be used to monitor response to treatment and can confirm relapse or indicate disease progression.

FISH studies for **TCRAD** for abnormalities of 14q32, **BCR-ABL1** to look for amplification of 9q34 as well as **KMT2A** for rare rearrangements of 11q23 will be carried out in the presence of a failed or normal karyotype.

**T-PLL**

T-cell prolymphocytic leukemia (T-PLL) is a rare mature T-cell lymphoproliferative disorder. In approximately 90% of patients with T-PLL the disease is characterised by inv(14)(q11q32) or a variant t(14;14)(q11;q32). In addition chromosome 8 rearrangements and 11q abnormalities leading to the deletion of the ATM and MLL genes are recurrent.

There is no conventional G-banded service for T-PLL but FISH studies for **ATM, CEP 8 and TCRAD** will be carried out on all confirmed T-PLL samples.

| FISH Probe | Cytogenetic Abnormality | Disease |
|---|---|---|
| **BCR-ABL1** | **NUP214/ABL1 (cryptic)** | **T-ALL** |
| **KMT2A (MLL)** | **t(11q23;)** | **T-ALL** |

| | | |
|---|---|---|
| **ATM** | **del(11q22.3)** | **T-PLL** |
| **TCRAD** | **T(14q11:?)** | **T-PLL** |
| **CEP 8** | **Ploidy** | **T-PLL** |

## Non-Hodgkin's Lymphoma (NHL)

FISH analysis for NHL is carried out on uncultured fixed cells from peripheral blood sample or bone marrow aspirate.

Selection of appropriate FISH test(s) should be done in conjunction with clinical information, morphology, flow cytometry, histopathology and immunohistochemistry.

### FISH tests available:

| Probe | Abnormality | Sub-type of NHL |
|---|---|---|
| **IGH-CCND1** | **t(11;14)(q13;q32)** | **Mantle cell** |
| **IGH-BCL2** | **t(14;18)(q32;q21)** | **Follicular** |
| **IGH** | **t(14q32;?)** | **Non-specific** |

## High-grade Non-Hodgkin's lymphoma

FISH analysis for HG-NHL is carried out on uncultured fixed cells from peripheral blood sample or bone marrow aspirate or bone marrow smears.

This category includes diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma and B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

IGK and IGL to detect BL variants in the absence of IGH-MYC rearrangement

Selection of appropriate FISH test(s) should be done in conjunction with clinical information, morphology, flow cytometry and histopathology

### FISH tests available:

| FISH Probe | Abnormality |
|---|---|
| **MYC** | **t(8q24)** |
| **IGH-MYC** | **t(8;14)(q24;q32)** |
| **IGK and IGL** | **t(2;8), t(8;22)** |
| **BCL2** | **t(18)(q21)** |
| **BCL6** | **t(3q26)** |

## Chronic Lymphocytic Leukaemia (CLL)

Chronic lymphocytic leukemia (chronic lymphoid leukemia, CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes.

FISH analysis for CLL is carried out on uncultured fixed cells from peripheral blood sample or bone marrow aspirate.

All analysis on CLL samples is carried out by interphase FISH using the following probe panel:

## FISH tests available:

| FISH Probe | Abnormality |
|---|---|
| **TP53/CEP 17** | **del(17p13)** |
| **ATM** | **del(11q22.3)** |
| **DLEU/LAMP/CEP12** | **del(13q)/trisomy 12** |

# Multiple myeloma (MM) plasma cell leukaemia

Multiple myeloma is a plasma cell disorder. CD138 positive cell selection is used to perform FISH studies at diagnosis and relapse only.

MGUS and monitoring samples are not accepted.

The current myeloma FISH panel contains the minimum diagnostic information required for risk stratification.

**Reference**: International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014 Nov;15(12):e538-48.

## FISH tests available:

| Probe | Abnormality |
|---|---|
| **CKS1B/CDKN2C** | **1q21 amplification/1p32 deletion** |
| **TP53/CEP17** | **del(17p)** |
| **IGH-FGFR3** | **t(4;14)** |
| **IGH-MAF** | **t(14;16)** |