Title: SE-HMDS Oncology Cytogenetics User Guide
Subject: Investigations performed in SE-HMDS Cytogenetics

Version number	4.0
Author	Fran Aldridge
Authorised by	Remi Oke
Issued on	December 2023

Contents

1. Introduction

Synnovis 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 SE-HMDS 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 SE-HMDS 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 Synnovis Cytogenetics laboratory at SE-HMDS is a UKAS accredited medical laboratory, no. 9092.

2. Contact Details

Correspondence Address:
Synnovis Cytogenetics (SE-HMDS)
Ground Floor, Hambleden Wing
King's College Hospital
Denmark Hill
London SE5 9RS

Sample Address:
King's SE-HMDS 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 7637

3. Main Departmental Contacts

Head of Laboratory

Fran Aldridge DipRCPath frances.aldridge@nhs.net

Tel 0203 299 7636

Operations Leads/Principal Clinical Scientists

Remi Oke remi.oke1@nhs.net

Zoë Thorn DipRCPath zoe.thorn@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

A Bone Marrow Aspirate (BMA) is the tissue of choice to investigate patients suspected of having leukaemia or related haematological neoplasms. Peripheral Blood (PB) can be sent if disease cells are present in sufficient numbers to allow cell culture. Peripheral blood is suitable for diagnoses of Chronic Lymphocytic Leukaemia (CLL) or T-cell prolymphocytic leukemia (T-PLL).

N.B. BMA should be sent in lithium heparin or sodium heparin vacutainers.

Please DO NOT use other anticoagulants such as EDTA, which may inhibit cell division as required for G-banded chromosome analysis. 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 suitable condition to be processed and analysed.

World Health Organisation (WHO) Guidance (2021)[1] states that: "Shippers of infectious substances shall ensure that packages are prepared in such a manner that they arrive at their destination in good condition and present no hazard to persons or animals during transport."

Similarly, under various dangerous goods transport/carriage regulations[2,3], 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.

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

6.1 Packaging requirements

Potentially infectious samples from GPs transported by designated vehicles provided by Synnovis or the local NHS Trust must be carried out in compliance with the UK and European road transport regulations[2].

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 packing Instruction 650 of ADR as follows:

- All samples in containers (e.g. tube, pot known as the "primary") must be placed in individual sample bags to avoid cross contamination. **Never send samples from different patients in the same 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 toweling 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 Synnovis 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.

At least 4ml of BMA sample where possible is required for successful cytogenetic studies. It is particularly important that BMA samples for successful Myeloma FISH studies are part of the first draw of the aspirate; as recommended by the European Myeloma Network[4].

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. BMAs from patients with suspected Myeloma need to arrive in the

laboratory before 10am on a Friday to allow for the lengthy processing procedure required for CD138+ cell selection before the weekend. As recommended by the European Myeloma Network[4].

6.2 Request/Referral Forms

Please use the King's SE-HMDS request form which may be retrieved from the South East Genomics website:
https://southeastgenomics.nhs.uk/glh/cancer-tests/haemato-oncology/

The reason for referral is important to determine which culture type or other processes 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 SE-HMDS.

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)
SARS-CoV-2
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 Synnovis 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

SE-HMDS Cytogenetics does not issue reports by fax.

7.3 Posting / E-mailing Reports

Full copies of authorised reports are emailed as PDFs using a group nhs.net account and can be sent by post to external referrers if necessary. 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 SE-HMDS, 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

The following table contains turnaround times (TATs) in calendar days. These are within NHSE guidelines, with some TATs that are more stringent due to local agreement*. See section 10 for further details of specific testing for each clinical scenario.

Clinical Scenario	Tests	TAT from test initiation	Priority
Diagnostic ALL or MPAL	*BCR*::*ABL1* FISH	3 days*	Very Urgent
	Appropriate reflex testing (see section 10).	14 days	Urgent
Diagnostic AML	APL FISH (*PML*::*RARA*/ *RARA*::)	3 days	Very Urgent
	AML FISH panel (≤80 years)* *when eligible for treatment with Gemtuzumab Ozogamicin (Mylotarg®)11 or CPX-351 (Vyxeos®)12	4 days*	Very urgent (local agreement)
	AML FISH panel (>80 years old)	14 days	Urgent
	Karyotype	14 days	Urgent
?Burkitt Lymphoma	*MYC* translocation FISH	3 days	Very Urgent
?other High-grade Lymphoma	High grade lymphoma FISH panel (see section 10)	5 days*	Urgent
?CML (strong indication)	*BCR*::*ABL1* FISH (if not previously confirmed by RT-PCR)	3 days	Urgent
	Karyotype	14 days	Urgent
Acute Leukemia with concerns of relapse	Karyotype and/ or FISH as appropriate	14 days	Urgent
CML in transformation	Karyotype	14 days	Urgent

Routine monitoring of AML, ALL, CML	As appropriate	21 days	Routine
MDS	See section 10	21 days	Routine
Myeloma	Myeloma FISH panel (See section 10)	21 days	Routine
CLL	FISH	21 days	Routine
Low grade Lymphoma	FISH as appropriate	21 days	Routine
Other		21 days	Routine
NHSE state that 90% of the samples should be reported within the guideline time (calendar days). 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[5].

BMA and PB samples referred to SE-HMDS Cytogenetics are disposed 3 months after receipt of a sample. 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

All cytogenomic investigations and reporting of cytogenomic findings are guided by established best practice guidelines listed in the references [6,7,8,9,10,11,12]

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) 2020[13].

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 SE-HMDS 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:

Referral Indication	Investigations	TAT
?CML	• **Urgent FISH** for *BCR::ABL1* if high suspicion and not already confirmed by RT-PCR	3 days
	• Full **karyotype** on BMA	14 days
Thrombocytosis/ polycythaemia/ ?MPN/ ?ET/ ?PRV	• Karyotype analysis in **NOT** routinely performed for MPN referrals.[14] • Studies to exclude *BCR*::*ABL1* are NOT routinely performed by FISH where CML is not strongly suspected. This can be done by RT-PCR as offered by the Laboratory for Molecular Haemato-Oncology (LMH) in SE-HMDS.	α
	• A **karyotype** study of a BMA will be performed if morphological studies indicate **≥5% of blasts**.	
	○ 5-9% blasts	21 days
	○ ≥10% blasts	14 days
	• Activation of Single nucleotide polymorphism array (**SNP-A**)* performed by the LMH in SE-HMDS for ○ **PMF (see below)** ○ **Post-PV/post-ET MF (see below)**	α

?Myelofibrosis	• If **<5% blasts** by morphological studies activation of **SNP-A*** performed by the LMH in SE-HMDS.	
	• A **karyotype** study of a BMA will be performed if morphological studies indicate **≥5% of blasts**.	
	○ 5-9% blasts	21 days
	○ ≥10% blasts	14 days
CML post treatment	• 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 LMH in SE-HMDS‡.	α
	• Aligned with ELN recommendations[15] the laboratory will perform a **karyotype** study of a BMA in the following scenarios	
	○ **Treatment failure/resistance** to exclude additional chromosome abnormalities (ACA).	14 days
	○ Progression or suspected progression to CML in **accelerated phase** or **blast phase.**	14 days
	• **FISH** (*BCR*::*ABL1*) monitoring may be appropriate in rare instances of atypical transcripts that cannot be measured by qPCR	21 days
Leukaemic Transformation (non-CML MPN → AML)	• **Secondary AML FISH panel** ○ -5/ del5q ○ -7/ del7q ○ del(17p); *TP53* deletion ○ *MECOM* (3q26) rearrangements	14 days Ω
	• Full **karyotype** on BMA	14 days

α Refer to LMH Laboratory User's Handbook for TATs
* SNP-A 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.
‡This test utilises RT-PCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations"
Ω FISH reported in advance of G-banding (4 day TAT) if clinically significant result

10.2 Eosinophilia

Standard investigations:

Referral Indication	Investigations	TAT
Hypereosinophilia (eos count ≥ 1.5 × 10^9/L) ?cause	• **Eosinophilia FISH panel** for rearrangements of ○ *FIP1L1*::*PDGFRA* (4q12) ○ *PDGFRB* (5q32) ○ *FGFR1* (8p11) ○ *JAK2* (9p24) ○ *ABL1* (9q34)	21 days
	• Full **karyotype** on BMA only[16].	21 days
Monitoring of previously detected gene fusion.	• Monitoring of *FIP1L1*::*PDGFRA* transcripts is available via the LMH in SE-HMDS‡.	α
	• Monitoring by FISH can be done for other rare fusions where molecular methods are not available.	21 days

α Refer to LMH Laboratory User's Handbook for TATs
‡This test utilises RT-PCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations"

10.3 Myelodysplastic/myeloproliferative overlap neoplasms

MDS/MPN standard investigations:

Referral Indication	Investigations	TAT
?CMML, MDS/MPN or ?atypical CML	• **FISH** to exclude ***BCR*::*ABL1*** [t(9;22)(q34;q11.2)] for CMML only when not done previously or concurrently by RT-PCR.	21 days
	• If **<5% blasts** by morphological studies activation of **SNP-A*** performed by the LMH in SE-HMDS	α
	• Full **karyotype** on BMA if **>5% blasts** by morphological studies (e.g. CMML-1/2)	
	○ 5-9% blasts	21 days
	○ ≥10% blasts	14 days
Monitoring	• Some monitoring by FISH as indicated to detect diagnostic chromosomal abnormality	21 days

Leukaemic Transformation (CMML → AML)	• **Secondary AML FISH panel** ○ -5/ del5q ○ -7/ del7q ○ del(17p); *TP53* deletion ○ *MECOM* (3q26) rearrangements	14 days Ω
	• Full **karyotype** on BMA	14 days

α Refer to LMH Laboratory User's Handbook for TATs
Ω FISH reported in advance of G-banding (4 day TAT) if clinically significant result
* SNP-A 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.

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 SE-HMDS]; 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.

MDS standard investigations:

Referral Indication	Investigations	TAT
?MDS/ thrombocytopenia/ neutropenia/ aplastic anaemia/ ITP ?t-MDS	• If **<5% blasts** by morphological studies activation of **SNP-A*** performed by the LMH in SE-HMDS in the absence of a prior condition indicating possible therapy-related myeloid neoplasm. • Fixed cytogenetic preparations will be stored for 6 months if a G-banded karyotype is necessary to confirm SNP-A findings or to clarify a IPSS-R cytogenetic risk category[17]	α
	• Full **karyotype** on **BMA** if **>5% blasts** by morphological studies **OR** a prior condition indicating possible **therapy-related** myeloid neoplasm.	
	○ 5-9% blasts	21 days
	○ ≥10% blasts	14 days
	• Those with a **failed** karyotype will have a **high risk MDS (translocation) FISH panel** performed to detect balanced rearrangements ○ *MECOM* (3q26) rearrangements	As above

	○ t(6;9)(p23;q34.1) [*DEK*::*NUP214*] ○ t(8;21)(q21.3;q22.1) [*RUNX1*::*RUNX1T1*] ○ t(?;21); other *RUNX1* rearrangements ○ *KMT2A* (11q23) rearrangements including t(9;11)(p21.3;q23.3) [*KMT2A*::*MLLT3*] ○ inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) [*CBFB*::*MYH11*] …and a SNP-A* will also be activated where possible unless: ▪ The patient is known to have previously received a bone marrow transplant. ▪ In ?t-MDS where the original clonal disorder is persistent within the bone marrow. Where a SNP-A is not feasible FISH for chromosomes 5 and 7 and *TP53* will also be performed.	
MDS Monitoring	○ Monitoring SNP-A/karyotype/FISH as indicated to detect diagnostic chromosomal abnormality	21 days
?Leukaemic Transformation/ increased blasts/ ?progression (MDS → ?AML)	• **Secondary AML FISH panel** ○ -5/ del5q ○ -7/ del7q ○ del(17p); *TP53* deletion ○ *MECOM* (3q26) rearrangements	14 days Ω
	• **Full karyotype** on BMA	14 days

α Refer to LMH Laboratory User's Handbook for TATs
Ω FISH reported in advance of G-banding (4 day TAT) if clinically significant result
* SNP-A 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.

10.5 Acute myeloid leukaemia

AML standard investigations:

Referral Indication	Investigations	TAT
APL suspected	• Rapid Very Urgent FISH for *PML*::*RARA* [t(15;17)] • FISH for other *RARA* (17q21.2) rearrangements that can be associated variant APL[18]	3 days (target <24 hours)

	○ Rearrangement of *RARA* not with *PML* will be investigated further by expedited karyotype studies to identify a translocation partner if possible. ○ Normal results will be followed by the appropriate AML FISH panel (see below).	
Diagnostic *de novo* AML patient age ≤80 years (eligible for treatment with Gemtuzumab Ozogamicin (Mylotarg®)[19] or CPX-351 (Vyxeos®)[20]:	• Very **Urgent Full AML FISH panel** performed to detect: ○ *MECOM* (3q26) rearrangements ○ -5/del5q ○ t(6;9)(p23;q34.1) [*DEK*::*NUP214*] ○ -7/del7q ○ t(8;21)(q21.3 q22.1) [*RUNX1*::*RUNX1T1*] ○ *KMT2A* (11q23) rearrangements including t(9;11)(p21.3;q23.3) [*KMT2A*::*MLLT3*] ○ t(9;22)(q34;q11.2); [*BCR*::*ABL1*] ○ inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) [*CBFB*::*MYH11*] ○ del(17p) [*TP53* deletion] ○ *RARA* (17q21.2) rearrangements	4 days
	• For referrals with **prominent eosinophils** in the absence of other disease defining cytogenetic findings by FISH add the **Eosinophilia FISH panel** for rearrangements of ○ *FIP1L1*::*PDGFRA* (4q12) ○ *PDGFRB* (5q32) ○ *FGFR1* (8p11) ○ *JAK2* (9p24) ○ *ABL1* (9q34)	14 days
	• **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 days
Diagnostic *de novo* AML patient age >80 years OR t-AML:	• **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 days
	• **FISH** for **balanced rearrangements** that may be cryptic or subtle by karyotype analysis. ○ *MECOM* (3q26) rearrangements ○ *KMT2A* (11q23) rearrangements including t(9;11)(p21.3;q23.3) [*KMT2A*::*MLLT3*] ○ inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB*::*MYH11* ○ t(?;17)(?;q21); *RARA* rearrangement	14 days

	• Those with a **failed** karyotype will have further FISH studies for the following: ○ -5/del5q ○ t(6;9)(p23;q34.1) [*DEK*::*NUP214*] ○ -7/del7q ○ t(8;21)(q21.3 q22.1) [*RUNX1*::*RUNX1T1*] ○ t(9;22)(q34;q11.2); [*BCR*::*ABL1*] ○ del(17p) [*TP53* deletion]	
Diagnostic secondary AML	• **Secondary AML FISH panel** ○ -5/ del5q ○ -7/ del7q ○ del(17p); *TP53* deletion ○ *MECOM* (3q26) rearrangements	14 days Ω
	• **Full karyotype** on BMA	14 days
Post treatment AML for monitoring	• **Transcript RNA based quantitative testing‡** is available via LMH at SE-HMDS for the following. ○ ***PML*::*RARA*** ○ ***RUNX1*::*RUNX1T1*** ○ ***CBFB*::*MYH11*** ○ ***BCR*::*ABL1*** ○ ***NPM1*** mutant Cytogenetic studies are therefore **not required** for these patients.	α
	• Monitoring karyotype/ FISH is available as indicated to detect diagnostic chromosomal abnormality	21 days
Relapsed AML	• **Full karyotype** *If no previous cytogenomic testing, case will be treated as per diagnosis	14 days
	• **FISH** as indicated to detect diagnostic abnormality/additional abnormalities	14 days

α Refer to LMH Laboratory User's Handbook for TATs
‡This test utilises RT-PCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations"

10.6 Acute leukaemias of ambiguous lineage

MPAL standard investigations:

Referral Indication	Investigations	TAT
Diagnostic MPAL	• MPAL FISH panel ○ *BCR*::*ABL1* [t(9;22)(q34;q11.2)] ○ *KMT2A* (11q23) rearrangements	3 days
	• Full karyotype	14 days
Post treatment MPAL for monitoring	• **Transcript RNA based quantitative testing‡** is available via LMH at SE-HMDS for the following. ○ ***PML*::*RARA*** ○ ***RUNX1*::*RUNX1T1*** ○ ***CBFB*::*MYH11*** ○ ***BCR*::*ABL1*** ○ ***NPM1*** mutant Cytogenetic studies are therefore **not required** for these patients.	α
	• Monitoring karyotype/ FISH is available as indicated to detect diagnostic chromosomal abnormality	21 days
Relapse MPAL	• **Full karyotype** *If no previous cytogenomic testing, referral will be treated as per diagnosis	14 days
	• **FISH** as indicated to detect diagnostic abnormality/ additional abnormalities	14 days

α Refer to LMH Laboratory User's Handbook for TATs
‡This test utilises RT-PCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations"

10.7 Precursor lymphoid neoplasms

10.7.1 ALL

ALL standard investigations:

Referral Indication	Investigations	TAT
Diagnostic B-ALL patient age >40 years old	• **B-ALL >40 FISH panel** ○ *BCR*::*ABL1* [t(9;22)(q34;q11.2)] ○ *KMT2A* (11q23) rearrangements ▪ If *KMT2A* rearranged, reflex testing to determine partner including *KMT2A*::*AFF1* [t(4;11)]	3 days 14 days
	• If initial FISH is not informative then reflex testing is initiated with the **Ph-like ALL FISH panel** to look for further recurrent abnormalities seen in adult ALL[21] ○ ABL-class fusions ▪ *ABL1* (9q34) ▪ *ABL2* (1q25.2) ▪ *PDGFRB*,*CSF1R* (5q32) ○ JAK-STAT fusions ▪ *CRLF2* (Xp22.33,Yp11.2) ▪ *JAK2* (9p24)	14 days
	Activation of Single nucleotide polymorphism array (**SNP-A**)* performed by the LMH in SE-HMDS allows detection of microdeletions associated with B-ALL and also when interrogated along with FISH results can identify low hypodiploidy or near-haploidy.	α
Diagnostic B-ALL patient age ≤40 years old	• **B-ALL ≤40 FISH panel** ○ *BCR*::*ABL1* [t(9;22)] ○ *KMT2A* (11q23) ▪ If rearranged *KMT2A*::*AFF1* [t(4;11)] ▪ If not t(4;11) – karyotype analysis ○ *ETV6*::*RUNX1* [t(12;21)] ○ *TCF3*::*PBX1* [t(1;19)] ○ *TCF3*::*HLF* [t(17;19)]	3 days 14 days
	• If initial FISH is not informative then reflex testing is initiated with the **Ph-like ALL FISH panel**	14 days

	○ **ABL-class fusions** ▪ *ABL1* (9q34) ▪ *ABL2* (1q25.2) ▪ *PDGFRB*,*CSF1R* (5q32) ○ **JAK-STAT fusions** ▪ *CRLF2* (Xp22.33,Yp11.2) ▪ *JAK2* (9p24)	
	• Activation of Single nucleotide polymorphism array (**SNP-A**)* performed by the LMH in SE-HMDS allows detection of microdeletions associated with B-ALL and hyperdiploidy, low hypodiploidy or near-haploidy.	α
Diagnostic T-ALL	• **T-ALL FISH panel** ○ *KMT2A* (11q23) rearrangements. ○ ABL-class fusions ▪ *ABL1* (9q34) ▪ *ABL2* (1q25.2) ▪ *PDGFRB*,*CSF1R* (5q32)	14 days
	• Activation of Single nucleotide polymorphism array (**SNP-A**)* performed by the LMH in SE-HMDS	α
ALL monitoring	• Cytogenetic studies are not usually appropriate for monitoring purposes in ALL.	
	• Quantitative detection of *BCR*::*ABL1* [t(9;22)] is offered by the LMH in SE-HMDS‡.	α
ALL relapse	• If no previous cytogenomic testing, referral will be treated as per diagnosis.	14 days
	• **FISH** and/ or **SNP-A** as indicated to detect diagnostic abnormality/ additional abnormalities	14 days/ α

α Refer to LMH Laboratory User's Handbook for TATs
‡This test utilises RT-PCR methods (RNA based) so samples must be < 72 hours old when received – refer to the LMH Laboratory User's Handbook Section 8: "Additional Information on Molecular Investigations"

10.8 Mature Lymphoid neoplasms

10.8.1 Chronic Lymphocytic Leukaemia (CLL)

CLL standard investigations:

Referral Indication	Investigations	TAT
CLL Diagnosis & Monitoring (every 12 months)	• SNP-A* performed by the Laboratory for Molecular Haemato-Oncology (LMH) in SE-HMDS	α
	• Lymphoid gene panel including *TP53* mutation performed by LMH in SE-HMDS	α
?Richter's transformat-ion	• HGL FISH panel to detect: ○ *MYC* (8q24.1) rearrangement ○ *IGH*::*MYC* [t(8;14)(q24.1;q32)] ○ *BCL2* (18q21) rearrangement ○ *BCL6* (3q27) rearrangement ▪ Reflex testing of *IGK*::*MYC* / *IGL*::*MYC* if *MYC* rearranged but not with *IGH*	5 days

α Refer to LMH Laboratory User's Handbook for TATs

10.8.2 Plasma cell Neoplasms

Please note: Before Myeloma FISH testing can be initiated referring clinicians must provide the Cytogenetics laboratory with the results of morphological investigations, immunophenotyping results and/ or histopathology results that clearly indicate the presence of clonal plasma cells at a level indicative of Plasma cell (multiple) Myeloma or Smouldering Myeloma (≥10%) [if not requested to be performed within SE-HMDS]; failure to provide this information will result in the sample being delayed. Fixed CD138+ selected 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.

Cytogenetics is not currently performed for Monoclonal Gammopathy of Uncertain Significance (MGUS), monitoring samples or those samples where a diagnosis of Myeloma is not evident from other SE-HMDS studies.

If external trephine results indicate a clear diagnosis of Myeloma, Myeloma FISH studies can be reinstated by referrers provided CD138+ selected cells are available.

Plasma cell neoplasm standard investigations:

Referral Indication	Investigations	TAT
Diagnosis of Plasma cell Myeloma/ Smouldering Myeloma (≥10% plasma cells in the bone marrow)	FISH studies have been selected to enable the detection of cytogenetic aberrations associated with an adverse outcome[22, 23] • Myeloma FISH panel on CD138+ selected plasma cells. ○ *TP53* (17p13) deletion ○ *CDKN2C* (1p32) deletion ○ *CKS1B* (1q21) gain ○ *IGH* (14q32.3) rearrangement ○ *MYC* (8q24.1) rearrangement Where *IGH* (14q32.3) is rearranged, sequential reflex FISH for: ○ t(4;14)(p16.3;q32); *IGH*::*FGFR3* ○ t(11;14)(q13;q32); *IGH*::*CCND1* ○ t(14;16)(q32;q23); *IGH*::*MAF* ○ t(14;20)(q32;q12); *IGH*::*MAFB* ○ t(6;14)(p21;q32); *IGH*::*CCND3*	21 days
	• Where a *TP53* deletion is identified by FISH, for transplant eligible patients aged 75 or below (where surplus CD138+ve cells are available); CD138+ve cells will be forwarded to LMH in SE-HMDS for *TP53* mutation testing as part of the Lymphoid gene panel. This allows the identification of High risk "double-hit"[23] patients.	α
Myeloma Relapse or progression (Smouldering -> MM)	• FISH on CD138+ selected cells for: ○ *TP53* (17p13) deletion ○ *CDKN2C* (1p32) deletion ○ *CKS1B* (1q21) gain ○ Diagnostic *IGH* t(14;v) rearrangement when present	21 days
	• Those referrals without known prior successful diagnostic cytogenetic FISH results will be tested as per diagnostic strategy.	

10.8.3 B-cell Non-Hodgkin Lymphoma (B-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:

Referral Indication	Investigations	TAT
?Burkitt Lymphoma	• Initial FISH panel to detect ○ *MYC* (8q24.1) rearrangement ○ *IGH*::*MYC* [t(8;14)(q24.1;q32)]	3 days
	• Subsequent FISH panel to detect ○ *BCL2* (18q21) rearrangement ○ *BCL6* (3q27) rearrangement ○ *+/-* Testing of *IGK*::*MYC* / *IGL*::*MYC* if *MYC* rearranged but not with *IGH*	5 days
High-grade B-NHL/ ?DLBCL	• HGL FISH panel to detect: ○ *MYC* (8q24.1) rearrangement ○ *IGH*::*MYC* [t(8;14)(q24.1;q32)] ○ *BCL2* (18q21) rearrangement ○ *BCL6* (3q27) rearrangement ○ *IGH*::*CCND1* [t(11;14)(q13;q32)] • Reflex testing of *IGK*::*MYC* / *IGL*::*MYC* if *MYC* rearranged but not with *IGH*	5 days
?Mantle cell Lymphoma	• FISH to detect: ○ *IGH*::*CCND1* [t(11;14)(q13;q32)] ▪ *TP53* (17p13) deletion (if *IGH*::*CCND1* detected) • Lymphoid gene panel including *TP53* mutation performed by LMH in SE-HMDS • *CCND3* FISH study available at request.	21 days α
?Follicular Lymphoma	• FL FISH panel at diagnosis (not required at staging) ○ *BCL2* (18q21) rearrangement ○ *BCL6* (3q27) rearrangement	21 days
?Splenic Marginal Zone Lymphoma	• FISH for deletion of 7q available at request.[24]	21 days

10.8.4 Mature T-cell neoplasms

Where indicated FISH analysis can be carried out on uncultured fixed cells from a peripheral blood sample or bone marrow aspirate

PHA-stimulated cultures are used where karyotype analysis is indicated. Selection of appropriate cytogenetic test(s) will be performed in conjunction with clinical information/request, morphology, flow cytometry, histopathology and immunohistochemistry.

Referral Indication	Investigations	TAT
?T-PLL	• Karyotype analysis of PHA-stimulated cultures from peripheral blood – This allows the detection of both major and minor criteria used to establish the diagnosis of T-PLL including the rare t(X;14)[25]	21 days
	• FISH panel to detect disease defining criteria ○ *TCL1A* rearrangement (14q32) ○ Deletion of 11q (*ATM*) ○ Detection of gain of 8q (*MYC*)	21 days
?Hepatosplenic T cell Lymphoma	• FISH for isochromosome 7q available at request.	21 days
Adult T-cell leukaemia/lymphoma (ATL)	• FISH for *TRA/D* (14q11) and *TP53* (17p13).	21 days

10.9 Histiocytic cell neoplasms

FISH analysis for Langerhans cell histiocytosis (LCH) is generally carried out on formalin-fixed, paraffin-embedded (FFPE) tissue block sections pre-processed in the histopathology laboratory at King's College Hospital. **Please note that this testing is currently not UKAS accredited.**

Standard investigations:

Referral Indication	Investigations	TAT
Histiocytosis	• FISH to detect: ○ *ALK* (2p23) rearrangement ○ *BRAF* (7q34) rearrangement ○ *NTRK1* (1q23) rearrangement	21 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. Guidance on regulations for the transport of infectious substances 2021-2022. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. **[PDC-HAE-CYT-TRANSREG]**
2. European Agreement concerning the International Carriage of Dangerous Goods by Road 2023 (ADR 2023) Volumes 1 & 2 **[PDC-HAE-CYT-ADR]**
3. The Carriage of Dangerous Goods and Use of Transportable Pressure Equipment Regulations (2009) as amended 2011. **[PDC-HAE-CYT-DGOODS]**
4. Fiona M. Ross, et al., Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. *Haematologica* 2012;97(8):1272-1277 https://doi.org/10.3324/haematol.2011.056176. **[PDC-HAE-CYT-EURMM]**
5. The Royal College of Pathologists: The retention and storage of pathological records and specimens (5th edition). 2015 **[PDC-HAE-CYT-RCPATH]**
6. Hastings, R.J. *et al.* (2016) Guidelines for cytogenetic investigations in tumours. *Eur J Hum Genet.*, https://doi.org/10.1038/ejhg.2015.35 **[PDC-HAE-CYT-Tumour Guidelines]**
7. Rack, K.A. *et al*. (2019). European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. *Leukemia*, 33:1851-1867. **[PDC-HAE-CYT-EURREC]**
8. Deans, Z.C., Ahn, J.W., Carreira, I.M. et al. Recommendations for reporting results of diagnostic genomic testing. Eur J Hum Genet 30, 1011–1016 (2022). https://doi.org/10.1038/s41431-022-01091-0 **[PDC-HAE-CYT-ESHGREP]**
9. ACGS Haemato-oncology Best Practice guidelines (2007) v1.01 **[PDC-HAE-CYT-ACCHAEM]**
10. ACGS Chronic Myeloid Leukaemia & Other Myeloproliferative Neoplasms (2011) v1.00 **[PDC-HAE-CYT-ACCCMLMPD]**
11. ACGS Acute Myeloid Leukaemia & Myelodysplastic Syndromes (2012) v1.00 **[PDC-HAE-CYT-ACCAMLMDS]**
12. ACGS Acute Lymphoblastic Leukaemia (2011) v1.00 [**PDC-HAE-CYT-ACCALL**]
13. ISCN 2020: An International System for Human Cytogenomic Nomenclature (2020) Edited by: Jean McGowan-Jordan, Ros J. Hastings, Sarah Moore. https://doi.org/10.1159/isbn.978-3-318-06867-2. **[PDC-HAE-CYT-ISCN]**
14. Cross NCP, Godfrey AL, Cargo C, Garg M, Mead AJ; A British Society for Haematology Good Practice Paper. The use of genetic tests to diagnose and manage patients with myeloproliferative and myeloproliferative/myelodysplastic neoplasms, and related disorders. Br J Haematol. 2021 Nov;195(3):338-351. doi: 10.1111/bjh.17766. Epub 2021 Aug 18. PMID: 34409596. **[PDC-HAE-CYT-MPN]**
15. Cross, N.C.P., Ernst, T., Branford, S. *et al.* European LeukemiaNet laboratory recommendations for the diagnosis and management of chronic myeloid leukemia. *Leukemia* **37**, 2150–2167 (2023). https://doi.org/10.1038/s41375-023-02048-y **[PDC-HAE-CYT-CMLPROG]**

16. Butt, N.M., et al., (2017), Guideline for the investigation and management of eosinophilia. Br J Haematol, 176: 553-572 **[PDC-HAE-CYT-EOS]**
17. Greenberg PL, *et al.,* Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012 Sep 20;120(12):2454-65. doi: 10.1182/blood-2012-03-420489. Epub 2012 Jun 27. PMID: 22740453; PMCID: PMC4425443. **[PDC-HAE-CYT-IPSSR]**
18. Mannan A,. et al., Genotypic and Phenotypic Characteristics of Acute Promyelocytic Leukemia Translocation Variants. Hematol Oncol Stem Cell Ther. 2020 Dec;13(4):189-201. doi: 10.1016/j.hemonc.2020.05.007. **[PDC-HAE-CYT-APMLVAR]**
19. 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. **[PDC-HAE-CYT-GEM]**
20. 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. **[PDC-HAE-CYT-CPX]**
21. Moorman AV, et al., Prognostic impact of chromosomal abnormalities and copy number alterations in adult B-cell precursor acute lymphoblastic leukaemia: a UKALL14 study. Leukemia. 2022 Mar;36(3):625-636. doi: 10.1038/s41375-021-01448-2. Epub 2021 Oct 16. PMID: 34657128; PMCID: PMC8885405. **[PDC-HAE-CYT-UKALL14]**
22. Sonneveld P, et al., Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. Blood. 2016 Jun 16;127(24):2955-62. doi: 10.1182/blood-2016-01-631200. **[PDC-HAE-CYT-MMPROG]**
23. Walker BA, et al., High-risk, Double-Hit, group of newly diagnosed myeloma identified by genomic analysis. Leukemia. 2019 Jan;33(1):159-170. doi: 10.1038/s41375-018-0196-8. Epub 2018 Jul 2. PMID: 29967379; PMCID: PMC6326953**. [PDC-HAE-CYT-MMDH]**
24. Watkins AJ, *et al*., Splenic marginal zone lymphoma: characterization of 7q deletion and its value in diagnosis. J Pathol. 2010 Mar;220(4):461-74. doi: 10.1002/path.2665. PMID: 20077527. **[PDC-HAE-CYT-SPLENICMZL]**
25. Philipp B. Staber, et al; Consensus criteria for diagnosis, staging, and treatment response assessment of T-cell prolymphocytic leukemia. *Blood* 2019; 134 (14): 1132–1143. doi: https://doi.org/10.1182/blood.2019000402 **[PDC-HAE-CYT-TPLL]**