Cambridge University Hospitals
NHS Foundation Trust

HODS

Haematopathology and Oncology Diagnostic Service

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Phone: (01223) 217132 **Email:** add-tr.HODS@nhs.net (search 'HODS' on nhs.net directory)

Lab opening hours: 9.00 am – 5.30 pm Monday to Friday (closed Bank Holidays). For urgent requests outside these hours, please contact the HODS on-call Consultant Haematologist via the Cambridge University Hospitals switchboard tel: (01223) 245151

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Contact HODS

Main enquiry line

Phone: 01223 217132
Email: add-tr.HODS@nhs.net (search 'HODS' on nhs.net directory)

Please use this main enquiry line for all non-urgent enquiries including requests for reports, updates on specimens and request for more sample bags/request forms. For notification of urgent samples, or if samples will be arriving after standard working hours (especially on a Friday), please notify the laboratory directly on the numbers below.

Contact list

For operational, scientific or clinical queries please contact:

Role / Section	Phone	Named Contact / Email Address
Clinical Lead	01223 217791	Dr Anna Godfrey anna.godfrey1@nhs.net
Operational issues	01223 216296	Operational Lead: David Bloxham dave.bloxham@nhs.net
	01223 586546	Deputy Operational Lead: Dr Fiona Cullen fiona.cullen@nhs.net
Immunophenotyping Laboratory	01223 586546	Head of Section: David Bloxham dave.bloxham@nhs.net
East GLH Cytogenetics Laboratory (including requests for cytogenetics medium)	01223 348866	General enquiries: cuh.eastglh-haemonc-cyto@nhs.net
		Head of Section: Bridget Manasse bridget.manasse@nhs.net
East GLH Molecular Laboratory	01223 348866	General enquiries: cuh.eastglh-haemonc-molecular@nhs.net
		Head of Section (myeloid): Dr Ilenia Simeoni i.simeoni@nhs.net
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Haematopathology Consultants	01223 217017	Dr Caoimhe Egan caoimhe.egan@nhs.net
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	01223 348274	Dr Joy Staniforth joy.staniforth@nhs.net

Introduction

The Haematopathology and Oncology Diagnostic Service (HODS) at Cambridge University Hospitals is the Specialist Integrated Haematological Malignancy Diagnostic Service (SIHMDS) for the East of England. We provide a multi-parameter, integrated service including morphology, immunophenotyping and histopathology for the diagnosis and classification of haematological malignancies.

Our highly experienced HCPC-registered laboratory scientists work closely with a dedicated team of consultants from both a haematology and histopathology background. This integration ensures every specimen is assessed, tested, interpreted and reported to the highest standards, and also enables continued development of high-quality clinical testing in the rapidly progressing field of molecular pathology.

Samples with a suspected or known haematological malignancy will be received at the HODS specimen reception and assessed according to the clinical details and/or morphological and flow cytometry findings. For cases requiring cytogenetic or molecular testing, these will be performed in partnership with the East Genomic Laboratory Hub (GLH) located on the Cambridge University Hospital site from whom the services are commissioned by NHS England. The results of each diagnostic modality (morphology, flow cytometry, cytogenetics, molecular, histopathology) will be reported separately as they become available, and a final comprehensive integrated report will then be compiled within HODS by collating and interpreting all the results together. For haematological malignancies, HODS will classify cases according to the latest WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. This approach is essential for "improving outcomes guidance" (IOG) compliance (https://www.nice.org.uk/guidance/ng47), and facilitates accurate classification and prognostications of the disease, informing personalised therapies that target specific characteristics of individual cancer patients. The testing strategies employed within HODS are outlined in the section "What and when in haematopathology diagnostics".

The Haematopathology and Oncology Diagnostic Service (HODS) is a UKAS accredited medical laboratory No.10003. The laboratory has close connections and collaborations with national and international diagnostic centres. Information concerning the current UKAS scope of the HODS and East GLH laboratories is available online by searching 'UKAS 10003' or 'UKAS 8840', respectively.

This edition of the HODS booklet provides information on how different diagnostic pathways are applied, based on the clinical information provided on the request form and morphological assessment, and also how to reach us for questions or discussions.

How to Submit Samples

See appendix 1 for the HODS submission form for samples being sent from outside CUH, and instructions on how to request tests on Epic for internal samples. Sample requirements are listed under the relevant sections.

See appendix 3 for suggested bone marrow aspirate sampling guidance (including MRD sample requirements) currently in use in CUH. This is for reference only, as hospitals will have their own sampling guidance. However, the need for separate samples for flow cytometry, molecular genetics and cytogenetics is of increased importance now that the genomic laboratory is geographically separate to HODS, to avoid delays in obtaining results. It is also a common misconception that FISH requires collection in cytogenetics medium, whereas EDTA is preferable.

Solid tissue samples will be accepted for HODS review where the suspected histological diagnosis is a haematological malignancy. Specimen requirements are detailed in the Haematopathology section of this document; cases should always be sent with the original request form and an authorised local histopathology report including assessment of non-haematopoietic components and reason for referral to HODS.

If a solid tissue case has been previously reported at another SIHMDS and requires review at HODS, then all material and reports from the local and SIHMDS reporting centres should be sent, including bone marrow aspirate slides where appropriate and all supplementary reports (e.g. flow cytometry, cytogenetics/FISH, molecular) relevant to the case. Please ensure the consultant and hospital requesting the review are specified with the referral. Cases originating outside the HODS referral area should generally be reported through the appropriate local SIHMDS pathway prior to referral onto HODS.

Urgent samples

The laboratory must be notified by telephone of all cases that are deemed clinically urgent. Please also highlight this on the request form. These samples will receive priority and the findings phoned to the Consultant named on the request form (please provide a contact number as this makes direct contact easier). All samples arriving after standard working hours should be notified to the laboratory where possible within working hours. Instructions for couriers delivering out-of-hours samples are given in appendix 2.

If urgent morphology or immunophenotyping is needed outside of working hours, please contact the on-call HODS consultant via Cambridge University Hospital switchboard on (01223) 245151.

For out-of-hours histopathology samples (non-trephine) please refer to guidance from your local Histopathology Department regarding sample submission. For CUH users this can be found on the Histopathology section of the CUH portal (http://connect2/article/1376/Histopathology).

A limited cytogenetic service is provided on Bank Holidays by East GLH for very urgent cases and may be available at weekends on discussion. A molecular scientist may also be available at weekends to aid with urgent sample processing. Please contact the HODS on-call Consultant Haematologist if required.

Samples for external molecular MRD testing

All AML and ALL samples for molecular MRD testing that are received via HODS must state "FOR MOLECULAR MRD" on the request form, or tick the relevant box on the HODS request form (appendix 1). At least two bone marrow EDTA samples should be supplied and the first should be labelled with a "1st pull for MRD" sticker; please contact the laboratory to obtain more stickers. If peripheral blood is required to be sent for MRD in addition to bone marrow, this must also be stated on the request form.

MRD tests that require RNA extraction include (but are not limited to) *NPM1*, *BCR::ABL1* (p190), *PML::RARA*, *CBFB::MYH11* and *RUNX1::RUNX1T1*. These can usually be dispatched to external laboratories via the GLH without processing if they are received on Monday-Thursday, either on the same day as collection or if the collection date was the day prior and the sample is received before 1.30pm. Samples not received in this timeframe will require processing in the GLH prior to external dispatch and overall turnaround times will be delayed. An alternative is for the samples to be sent directly from the local hospital to the specialist MRD laboratory.

Peripheral blood samples for genomic tests only

Although HODS screens all bone marrow samples, this is not necessary for many peripheral blood samples because a blood film has been reviewed in the referring hospital, the diagnosis may already be established (e.g. CLL) and only genomic tests are required (e.g. MPN screening tests, TP53 mutations, CLL FISH). HODS will screen peripheral blood if the request form asks for flow cytometry or the clinical information implies the diagnosis is unknown e.g. "lymphocytosis ?cause". If only cytogenetic or molecular tests are requested, HODS will infer that the referring clinician has made an assessment of the clinical and laboratory information and has requested appropriate tests. Please note that myeloid NGS panel testing on peripheral blood is not generally recommended as a screening test for patients with unexplained cytopenias, since there is a risk of false negative results (due to low "tumour burden" in peripheral blood) and most patients with positive results will need definitive diagnostic assessment with bone marrow examination.

Please note that if a genomic test is performed by a specialist centre rather than East GLH (e.g. *BCR::ABL1* TKD mutation testing by NGS or AML MRD on peripheral blood), the sample can be sent directly to the appropriate GLH by the hospital/clinician.

Vitreous humour

These samples are very challenging due to their small volumes and often paucicellular nature. Haemato-oncological testing modalities are frequently non-diagnostic and it is important that the most appropriate sample is sent for the most important testing modality/modalities, in the context of the clinical differential diagnosis. In the setting of suspected primary intra-ocular lymphoma, possible HODS testing MUST be discussed with the flow cytometry team in advance of sampling.

Please note that samples for conventional cytomorphological interpretation ("cytology"), microbiological/virological assessment and cytokine testing are not handled by HODS and should be discussed with local providers of these services so that appropriate samples can be sent accordingly.

Specimen labelling

- Sample tubes and pots must be labelled with at least three points of identification, e.g. patient name (surname and forename) and two additional identifiers, such as date of birth, hospital number or NHS number.
- Slides must be labelled with the patient name (surname and forename), date of collection and one additional identifier.
- Addressograph labels may be used to label specimen tubes or pots, but not slides.
- The date and time of sample collection should be provided on the request form.
- If not an internal Epic order, then the request form should include details of the referring centre, relevant clinical information, the referring clinician's name and contact details.
- Different samples must be distinguished by labelling the pots/tubes, e.g. BM (bone marrow) or PB (peripheral blood), and 'first pull'.
- Insufficient or incorrect information on a specimen container(s) or request form can result in the sample being returned to the sender and delays in sample processing.

Packaging samples

Samples sent by Royal Mail or courier must comply with packaging instruction P650 for UN3373 biological substance, category B (Diagnostic specimens).

Packaging shall be of good quality, strong enough to withstand shocks (95 kPA pressure) and loadings normally encountered during carriage, including transhipment between vehicles or containers and between vehicles or containers and warehouse as any removal from a pallet or over pack for subsequent manual or mechanical handling. Packaging shall be constructed and closed to prevent any loss if contents that might be caused under normal conditions of carriage by vibration or by changes in temperature, humidity or pressure.

Packaging shall consist of three components:

- Primary receptacle
- Secondary packaging and
- Outer packaging

There should be sufficient absorbent material placed between the primary receptacle(s) and secondary packaging to absorb any spillage. The primary receptacle and absorbent material must be placed into a single leak-proof bag with the request form in the pouch. The package should be clearly labelled "BIOLOGICAL SUBSTANCE, CATEGORY B" and "UN3733" must be placed inside a diamond-shaped mark which has a minimum dimensions of 50 mm x 50 mm; the width of the line forming the diamond shall be at least 2 mm and the letters and numbers of UN3733 shall be at least 6 mm high. "BIOLOGICAL SUBSTANCE, CATEGORY B" shall also be at least 6 mm high and marked adjacent to the diamond-shaped mark.

For transportation around the Trust (Addenbrookes) they will not need any dangerous goods markings as the transport regulations do not apply.

Please note: UN3373 Biological Substance, Category B only applies to KNOWN or SUSPECTED infectious specimens. HODS treats all samples as potentially high risk therefore it is advised to use UN3373 Biological Substance, Category B.

HODS expected turnaround times (TATs)

Test	Turnaround Time	Target

Bone marrow aspirate morphology	3 working days	80%
Immunophenotyping	3 working days	80%
CD34 enumeration	2 hours	80%
Lymph node histology	5 working days	80%
Bone marrow trephine histology	6 working days	80%
Clinically urgent aspirate (suspected acute leukaemia or Burkitt lymphoma)	Verbal communication within 6 hours	90%
Integrated report	Six weeks	90%

For further information about the cytogenetic and molecular tests provided by the East GLH, please refer to their website: https://www.eastgenomics.nhs.uk/

Clinical details

Appropriate clinical details are essential to facilitating rapid and effective diagnosis. If any additional relevant information becomes available after sample submission, please email this to a member of the HODS reporting team. If comprehensive testing to provide prognostic information is not appropriate for a patient due to age or performance status, please provide this information in the clinical details and these standard diagnostic pathways will be adjusted accordingly. If patients are being monitored on a clinical trial, please also provide this information to avoid duplicate testing.

Sample testing frequency will be dictated by the specific clinical scenario. Reports will indicate if a sample is technically inadequate for diagnostic interpretation so that repeat testing would be required.

Consent prior to testing

Many samples investigated for a haematological malignancy will undergo further genomic tests including cytogenetics and/or next-generation sequencing (NGS) panels. The aim of these tests is to look for abnormalities relevant to this patient that explain the clinical presentation or provide further clinically useful information. These tests may also in a small minority of cases demonstrate unexpected potential germline findings which could have clinical significance for the patient and/or family members. In some cases additional testing or discussion with other clinical teams may be recommended.

It is recommended that patients undergoing investigation for a possible haematological malignancy should be counselled and at least verbal consent taken prior to genetic testing, to explain the possible results including the implications of identifying a germline mutation (Killick *et al*, Br J Haematol 2021;194(2):282-293).

Second opinions

Second opinions on selected cases from outside the contracted HODS region may be arranged with the prior approval of a HODS consultant. The consultant must be contacted by email in advance and a copy of the authorising email response should be enclosed with the case, together with the original report. Cases for second opinion which are received without prior approval may be returned to the submitting centre.

Morphology (Haematological Cytology)

Consultant Haematologists	Dr Anna Godfrey	01223 217791
	Dr Emma Gudgin	01223 256168
	Dr Pedro Martin-Cabrera	01223 274967
	Dr Emily Mitchell	01223 216616
	Dr Audrey Morris	01223 217073
	Dr Wai Keong Wong	
HODS Duty Scientists	David Bloxham	
	James Bowler-Barnett	
	Elaine Bradford	
	Hannah Creasey	
	Fiona Cullen	
	Cherise Wilton	

Screening

A morphological screening assessment (modified Wright's stain) is performed on almost every sample by the HODS duty scientist. Morphological screening alone may provide the diagnosis, and will direct the strategy of further investigations (e.g. choosing relevant flow cytometry and/or FISH panels or molecular tests). This screening can also be useful in assessing the quality of the sample and may indicate any possible limitations that may arise due to cellularity or integrity.

Bone marrow aspirates are reported by a consultant haematologist and where appropriate another consultant double-reports the case. When appropriate, a differential count is performed on peripheral blood and/or bone marrow aspirate slides. Morphological assessment will be undertaken to assess cellularity and the presence of myelodysplasia or other abnormal cells. Iron stain (Perls' Prussian blue) is performed if indicated.

Appropriate specimens

- Blood smear (unstained)
- Bone marrow smears x3 and/or squash (unstained)
- Bone marrow aspirate (1-2 mL) in EDTA
- Dab preparations from trephine or solid tissue (lymph node)
- Body fluids e.g. CSF; pleural fluid, in conservation fluid

Sample preparation & storage	Air-dried, room temperature. Samples that are not dried adequately prior to packaging often stain abnormally and are not suitable for full morphological assessment.
Bone marrow aspirate reporting	The bone marrow aspirate report will provide a description and quantify the different cell populations present in the sample. Abnormal cell types will be described in detail and myelodysplasia will be quantified per cell line, when indicated. Iron stain (Perls' Prussian Blue) is performed if indicated. Blood films should be included with all bone marrow aspirate samples. An interpretation of the morphological results will be provided, with a reference to the most pertinent pending investigations.

Immunophenotyping (Flow Cytometry)

Scientist in Charge: David Bloxham 01223 586546

Flow cytometry is performed primarily using 8 colour panels (progression to 12 colour panels is underway) in addition to the forward and side scatter properties of the cells. Antibody panels are designed to identify the main cell populations present in the sample and to focus on particular populations of interest with respect to lineage assignment and stage of differentiation. Aberrant antigen expression or asynchrony can be a marker of abnormality. Additionally, detection of light chain restriction within B-cell populations can be a very powerful tool for the detection of clonal B-lymphoid populations.

Bone marrow and tissue samples are usually reported with the percentage of a population of interest together with their phenotype. When reporting peripheral blood lymphoid populations this usually includes absolute values.

Specimens required

Blood (10ml EDTA) and/or bone marrow (1–2 mL EDTA) – this can be the sample provided for morphology:

- If sending peripheral blood or bone marrow, please enclose an unstained/stained smear preparation for evaluation
- If bone marrow aspirates are unobtainable or inadequate, bone marrow trephines (placed in sterile saline or RPMI) can be disaggregated for flow cytometric analysis*

Fresh tissue biopsy* or fine needle aspirate (FNA) in 1ml sterile saline or RPMI

Body fluids (in sterile tube) e.g. CSF, pleural fluid, lavage

Apheresis collection and/or cord blood in EDTA or ACD

***This must always be performed as a secondary investigation. The first bone marrow trephine biopsy or tissue biopsy must always be placed in formalin for primary haematopathology reporting.**

Sample preparation & storage

Samples must be analysed within 48 hours of collection and kept at $4^{O}C$ before despatch to preserve the expression of markers used for immunophenotyping; delayed processing may lead to unreliable or uninterpretable results. Please note this is particularly the case for material from fresh tissue biopsies or body fluids, which must be analysed as soon as possible after collection to maximise cell viability;

please phone the laboratory in advance when these samples are expected.

Reporting

The immunophenotyping report will include the clinical details from the request form and will state the sample type and cell population analysed.

The percentage of the cells with the phenotype of interest will be reported with respect to the whole population.

An overall interpretation and conclusion will be provided. Phenotypes, which may be useful for monitoring of minimal residual disease or response evaluation, will be indicated.

Haematopathology

Consultant Haematopathologists	Dr Lívia Rásó-Barnett (Specialty Lead)	01223 217791
	Dr Caoimhe Egan	01223 217017
	Dr John Grant	01223 216744
	Dr Elizabeth Soilleux (Honorary)	01223 254842
	Dr Joy Staniforth	01223 348274

HODS provide an integrated Haematopathology diagnostic service which covers histopathology, diagnostic cytology, immunohistochemistry and molecular pathology.

Specimen requirements

Specimen	Sample type	Specimen requirements
Bone marrow trephine	In formalin	Bone marrow trephine biopsies should be immersed in six to ten times the volume of neutral buffered formalin (10%) in an appropriately sized container and the lid secured properly. The pot should be labelled with a formalin hazard label.
	Slides/tissue blocks*	Fixed and stained sections with 10 unstained sections *or* Block(s) *or* Block(s) plus 20 consecutive serial sections (2–4 micrometres thin) with minimal trimming. Submitted as 3 H&E stained slides at level 1, 10 and 20 with the remaining 17 unstained slides for further IHC/retic.
Lymph node sample for flow cytometry		Please refer to section on Immunophenotyping for requirements. Arrange in advance with the Immunophenotyping section (01223 586546).
Lymph node sample (core biopsy, excisional biopsy or cell block from aspiration	Slides/tissue blocks	Submit single H&E stained slide from each block(s) plus block(s) *or*

cytology/EBUS-FNA) for histomorphology		Single H&E stained slide from each block(s) plus 20 unstained slides from each block(s) *Note:* also attach the original request form (or a copy), as well as the local report and information about work-up performed and material retained in the referring hospital. **Please note that HODS does not accept unprocessed non-trephine samples (other than fresh samples for flow cytometry). These will be returned to the referring centre for tissue processing.**
Splenic biopsy	Slides/tissue blocks	Block(s) plus 20 consecutive serial sections (2–4 micrometres thick) with minimal trimming Submitted as 3 H&E stained slides at level 1, 10 and 20 with the remaining 17 unstained slides for further IHC/retic

**For bone marrow trephine biopsy, local decalcification protocol to be followed. If local protocol is not in place, then the current protocol at department of Histopathology (Cambridge University Hospitals) can be followed: after fixation in 10% formalin (for 24 hours), bone marrow trephine biopsies undergo 24 hours EDTA decalcification (pH 6.5-7, at room temperature) before processing and sectioning.*

Solid tissue samples are stored in the CUH histopathology laboratory. Slides cut or stained in CUH will remain with CUH. After testing, the submitting centre's blocks and original slides are returned through the CUH Histopathology Archive Team; please contact HODS if material is required urgently. Additional fixed material may be stored for a period in the East genomics laboratory.

Workflow of bone marrow trephine biopsies

1. Bone marrow trephine biopsy samples in formalin should be sent to HODS (Level 3, Pathology Block) in purple HODS specimen bags alongside the liquid samples for morphology, flow cytometry, cytogenetic and molecular studies. They are booked in and linked via the specimen (SP) number in HODS.
2. After booking in, bone marrow trephines are transferred to the Department of Histopathology (LMB Cut-up room) for tissue processing, i.e., turning the sample from a tissue core in formalin to a tissue core in a paraffin block, enabling thin sections to be cut for slides.
3. Samples need to be fixed in six to ten times the volume of neutral buffered formalin (10%) in an appropriately-sized container for 24 hours. It is therefore of paramount importance that the referring clinical team clearly labels sampling time on the container.
4. Once 24 hours of fixation is complete, samples will be placed into EDTA (molecular decal solution) for 24 hours each evening Monday–Thursday and Friday–Saturday AM.

5. After 24 hours of decalcification is complete, specimens are processed overnight and then embedded the next morning for section cutting.
6. Once sections are cut, they are stained for H&E and transferred back to HODS for allocation to the Consultants in HODS.

Consultants request appropriate immunopanels and report cases once immunostained slides are available. For example, if a trephine sample is taken on a Tuesday before 3pm and reaches HODS by midday on Wednesday, HE stained slides should be available by Friday or Monday morning at the latest.

Potential factors delaying histopathology reports

1. Limited clinical information provided. Highlighting responsible clinician with email address and/or phone number is useful, but does not replace providing adequate clinical information upfront.

   As well as information on relevant linked clinical, radiological, and laboratory findings, details for non-marrow histological samples should ideally include:

   - *Biopsy site*, including *laterality* if applicable
   - Whether or not the patient has a *known current/previous haematological malignancy*
     - If there is a known current/previous haematological malignancy, the *WHO diagnostic entity or <u>at least</u> lineage* of this
   - Whether or not the patient has known *immune deficiency/dysregulation,* and if ascertained the underlying aetiology.
2. Limited sample size, for example EBUS-FNA samples or scanty material, as these need to be worked up in a stepwise fashion rather than with one diagnostic panel. A separate sample submitted for flow cytometry can greatly aid diagnostic yield.
3. Sample quality. Interpretation is more challenging in the presence of significant amounts of crush or cautery artefact and with small diameter tissue cores taken with higher gauge needles.

What and when in haematopathology diagnostics

These pathways list the tests that will be performed routinely by HODS and East GLH on samples based on the diagnostic information provided and HODS morphology screening of peripheral blood (PB) and bone marrow (BM) samples. If comprehensive testing to provide prognostic information is not appropriate for a patient due to age or performance status, please provide this information in the clinical details and these standard diagnostic pathways will be adjusted accordingly. If patients are being monitored on a clinical trial, then follow up samples may not be needed as they will be performed as part of the trial by external laboratories.

Tests additional to these standard pathways may also be available from East GLH; these can be requested in the clinical details or discussed with any member of the HODS team.

Myeloproliferative Neoplasms

At diagnosis:

Condition / Tests	MPN	CML
Morphology	Blast count / megakaryocytes Assessment of dysplasia Iron stain	Blast count / megakaryocytes Basophilia
Flow cytometry	If blasts > 5%	If blasts > 5%
FISH	Eosinophilia: • 4q12 *FIP1L1*::*PDGFRA* • 5q33 *PDGFRB* break-apart • 8p11 *FGFR1* break-apart • 9p24 *JAK2* break-apart • Consider *ETV6* break-apart • Consider 5q– in appropriate context	*BCR*::*ABL1*
Karyotype	Karyotyping may be considered in appropriate patients and is available upon request	t(9;22) Additional abnormalities
Molecular genetics	*JAK2* V617F mutation analysis If *JAK2* V617F mutation not detected, then: • *JAK2* exon 12 mutation analysis (in suspected PV) • *CALR* exon 9 and *MPL* exon 10 mutation analysis (in suspected ET or PMF) *KIT* D816V mutation analysis (in suspected systemic mastocytosis) *BCR*::*ABL1* fusion gene to exclude CML (if thrombocytosis, otherwise molecularly negative). Consider *FIP1L1*::*PDGFRA* fusion gene in appropriate context. Myeloid NGS in myelofibrosis if potential transplant candidate or if full prognostic information required	*BCR*::*ABL1* fusion gene diagnostic qualitative PCR and quantitative PCR

BM histology	Cellularity Megakaryocyte morphology Fibrosis CD34 / CD117	Cellularity Megakaryocyte morphology Fibrosis CD34 / CD117 (Trephine biopsy may not be necessary if good aspirate obtained)

Myeloproliferative Neoplasms

At follow up:

Condition / Tests	MPN	CML
Morphology	Blood cell counts	Blood cell counts Basophilia / megakaryocytes
Flow cytometry		
FISH		*BCR::ABL1*
Karyotype		t(9;22), additional abnormalities
Molecular genetics		*BCR::ABL1* E13A2/E14A2 transcript quantification For rare / unusual *BCR::ABL1* fusion transcript analysis, samples are sent to another GLH as per NHS England
BM histology	Cellularity Megakaryocyte morphology Fibrosis CD34 / CD117	

Myeloproliferative Neoplasms

At transformation (acceleration/blast phase):

Diagnosis / Tests	MPN	CML
Morphology	Blast count Dysplasia Iron stain (presence or absence of ringed-sideroblasts)	Blast count Basophilia

Flow cytometry	Blast phenotype	Blast phenotype
FISH	If appropriate	
Karyotype	If appropriate	t(9;22), (additional abnormalities)
Molecular genetics	Myeloid NGS if disease transformation in transplant candidate	*BCR::ABL1* TKD mutation analysis
BM histology	Fibrosis CD34 / CD117	Fibrosis CD34 / CD117

Acute Myeloid Leukaemia and Myelodysplastic Syndromes

At diagnosis:

Condition / Tests	Acute Myeloid Leukaemia	MDS; MDS/MPN Aplastic anaemia and other bone marrow failure disorders
Morphology	Blast count % of dysplasia per cell-lineage, if AML Maturation/differentiation, if AML Grade any underlying dysplasia Maturation/differentiation, if AML Iron stain (presence or absence of ring sideroblasts)	Blast count; monocyte count % of dysplasia per cell lineage Iron stain
Flow cytometry	Acute leukaemia panel Establish Leukaemia Associated Immunophenotype (LAIP)	If increased blasts on morphology Markers of aberrant myeloid maturation
FISH	May vary depending on clinical situation. If patients are potential candidates for remission induction chemotherapy: AML FISH panel (all cases): • t(8;21) • inv(16)/t(16;16) • chr 5 and 7 abnormalities • t(15;17) (will also detect i17q) • *KMT2A* (*MLL*) rearrangements • 17p if any other abnormalities NUP98 FISH is added if no other molecular MRD marker identified	Can be requested if rapid TAT of cytogenetic changes required, or only limited cytogenetic analysis needed. Panel: • -5/del5q • -7/del7q • Trisomy 8 • Del20q12 MECOM available as an additional probe
Karyotype	Recurrent translocations and other abnormalities	For IPSS. May use SNP array.
Molecular genetics	*FLT3* ITD and allelic ratio – rapid TAT *FLT3* TKD – rapid TAT *NPM1* and quantitation if positive	Consider testing as per AML depending on blast count and clinical factors.

	Myeloid NGS *PML::RARA* if t(15;17) detected by FISH *RUNX1::RUNX1T1* if t(8;21) detected by FISH *CBFB::MYH11* if inv(16) or t(16;16) detected by FISH *KMT2A* translocation partner if 11q23 abnormality detected by on FISH If other fusion identified (e.g. by RNA fusion panel), samples sent to Viapath at Guy's Hospital, London for baseline identification of MRD marker	
BM histology	May not be required if definite AML on PB and good aspirate sample. If ambiguous lineage leukaemia or aparticulate aspirate, trephine is required.	Cellularity Dysplasia Fibrosis CD34 / CD117

Acute Myeloid Leukaemia and Myelodysplastic Syndromes

At follow up:

Condition / Tests	Acute Myeloid Leukaemia	MDS; MDS/MPN
Morphology	Blood counts Blast count	Blood counts Blast count
Flow-cytometry	If LAIP established at diagnosis	
FISH	If marker to determine cytogenetic remission.	If marker to determine cytogenetic remission (patient treated with curative intent)
Molecular genetics	If molecular marker of disease, MRD assessment will be performed as appropriate Tests not performed in-house are sent to Viapath at Guy's Hospital, London laboratory	Consider if >6 months since last assessment, and presence of one or more of the following: • Clinical worsening

	Common tests are listed (rarer assays may be performed after discussion with Guy's MRD laboratory): • *NPM1* • *PML::RARA* • *RUNX1::RUNX1T1* • *CBFB::MYH11* • *KMT2A (MLL)* – fusion transcripts • *NUP98::NSD1* • *ETV::RUNX1*	• Morphological progression • Cytogenetic progression
BM histology	Blast "equivalent(s)" % May not be required in non-transplanted patient.	Dysplasia Fibrosis CD34/CD117

Acute Myeloid Leukaemia and Myelodysplastic Syndromes

At relapse:

Condition / Tests	Acute Myeloid Leukaemia	MDS; MDS/MPN
Morphology	Blast count Dysplasia	Blast count % of dysplasia per cell lineage Iron stain (presence or absence of ring sideroblasts)
Flow cytometry	Acute leukaemia panel	If blast > 20%
FISH	If marker at diagnosis	May be appropriate
Karyotype	(Additional) abnormalities	May be appropriate
Molecular genetics	As for diagnosis	May be appropriate
BM histology	CD34 / CD117 Dysplasia	Dysplasia, fibrosis, cellularity CD34 / CD117

Acute Lymphoblastic Leukaemia

At diagnosis:

Condition / Tests	Acute Lymphoblastic Leukaemia
Morphology	Blast count
Flow cytometry	Acute leukaemia panel Establish Leukaemia-Associated Immunophenotype (LAIP)
FISH	*B-ALL:* *BCR::ABL1, ETV6::RUNX1, KMT2A* If negative, *PBX1::TCF3, HLF::TCF3* If negative, *ABL* class rearrangements and other probes *T-ALL:* *MLL, BCR::ABL, p16, TRA/TRD, TRB, TRG, STIL, TLX3 (HOX11L2)*
Karyotype	SNP array
Molecular genetics	Confirm FISH findings *IgH/TCR* to be sent to appropriate laboratories to determine MRD marker depending on patient age RNA fusion panel (performed externally) considered on individual case basis if case would otherwise would be B-ALL NOS NGS panel including TP53 if hypodiploid ALL
BM Histology	Not required if diagnosis confirmed by flow in the PB or aspirate. If ambiguous lineage leukaemia in the PB or aspirate, trephine is required.

Acute Lymphoblastic Leukaemia

At follow up:

Condition / Tests	Acute Lymphoblastic Leukaemia
Morphology	Blast count
Flow cytometry	At every time point independent of presence of LAIP
FISH	To confirm cytogenetic remissions where appropriate
Karyotype	Not required

Molecular genetics	*IgH/TCR* to be sent to appropriate national MRD laboratories depending on patient age *BCR::ABL1* MRD or *KMT2A*::x depending on diagnostic findings – in adult patients these are monitored routinely rather than *IgH/TCR*
BM Histology	Not required routinely at follow up

Mature lymphoid neoplasms

At diagnosis:

Condition / Tests	LPD	MM/MGUS
Morphology	Lymphocyte and differential count Lymphocyte morphology Iron Stain if applicable	Plasma cell and differential count Iron Stain if applicable
Flow cytometry	LPD Screen Extended B and/or T panel as appropriate Light chain expression (κ/λ) if B lineage neoplasm	Plasma cell phenotype including cytoplasmic light chain expression
FISH	May be performed for relevant abnormalities in CLL, MCL, PLL, FL, Burkitt lymphoma	Deletion 1q21, additional 1p32, *TP53* deletion, *IGH::FGFR3, IGH::MAF, IGH::CCND1, IGH::MAFB*
Molecular genetics	In appropriate cases; • Hairy cell leukaemia: *BRAF* V600E mutation analysis • Lymphoplasmacytic lymphoma: *MYD88* L265P mutation analysis • Anaplastic large cell lymphoma: *NPM::ALK* fusion gene	

	• T-cell LPD / lymphoma: *TCR* gene rearrangement (clonality) • B-cell LPD / lymphoma: *IG* gene rearrangement • Chronic lymphocytic leukaemia: TP53 sequencing by NGS (or broader NGS panel) in pre-treatment samples • *IGVH* mutation status (on request)	
BM histology	Assessment of involvement and classification of disease FISH, if required – may be possible depending on disease	Extent of plasma cell involvement Plasma cell phenotype if not already conclusively determined by flow cytometry FISH, e.g. t(11;14), may be done if low level plasma cells in the liquid sample.

Mature lymphoid neoplasms

At follow up:

Condition / Tests	LPD	MM/MGUS
Morphology	Lymphocyte count Lymphocyte morphology	Plasma cell count
Flow cytometry	Specific B- or T-cell analysis to detect residual disease High resolution (0.01%) assays for CLL	Normally not indicated at follow-up in myeloma Post-autograft MRD Assessments can be performed upon request.
BM histology	Assess extent of residual lymphoma involvement	Extent of residual plasma cell involvement

Mature lymphoid neoplasms

At relapse:

Condition / Tests	LPD	MM/MGUS
Morphology	Lymphocyte count Lymphocyte morphology	Plasma cell count
Flow cytometry	If significant change in morphology	Normally not indicated in cases of clear relapse of myeloma
FISH	CLL FISH may be indicated	May be required to detect therapeutic target, e.g. t(11;14)
Molecular genetics	CLL TP53 sequencing by NGS (or broader NGS panel) may be indicated	
BM histology	Extent of lymphoma involvement Transformation	Extent of plasma cell involvement

Cytogenetics (Karyotyping and FISH)

Scientist in Charge: Bridget Manasse (01223) 348710

The East GLH cytogenetics laboratory works in partnership with HODS performing karyotyping using G-banding, FISH (Fluorescence *in situ* Hybridisation) and SNP array, and the results are integrated with other testing modalities by HODS. Enquires about samples, turn-around-times, and availability of results should be addressed to cuh.eastglh-haemonc-cyto@nhs.net. Please see also the GLH website: https://www.eastgenomics.nhs.uk/

Karyotyping using G-banding

Karyotyping using G-banding is performed on the metaphase spreads of cells cultured from bone marrow aspirate samples or peripheral blood collected in sterile bone marrow transport medium (available from the laboratory) or Lithium Heparin. This identifies chromosomal changes, which can be associated with a wide variety of haematological disorders and can be important for classification of the disease and give a guide to disease prognosis.

Single Nucleotide Polymorphism (SNP) array

Single Nucleotide Polymorphism (SNP) array is usually performed on DNA obtained from the aspirate sample, although if not available peripheral blood may be used. SNP arrays are particularly useful to detect copy number changes at a higher resolution than a conventional karyotype and were previously termed "molecular karyotype". It is also useful to detect copy number neutral loss of heterozygosity of particular regions of the genome. The sensitivity is approximately 20% and are often successful in demonstrating genetic changes where conventional karyotyping often failed.

Currently we use SNP arrays in the following cases:

- Acute lymphoblastic leukaemia
- Aplastic anaemia and PNH
- May be used in myelodysplastic disorders
- Where G-banding has failed for sample/technical reasons, but full chromosomal analysis is required

Fluorescence *in situ* hybridisation (FISH)

Fluorescence *in situ* hybridisation (FISH) identifies specific genetic changes using fluorescently labelled chromosomal probes. FISH can be performed as the primary genetic test, e.g. as a screening test for recurrent cytogenetic abnormalities in a suspected disease, in diseases that cannot easily be cultured (e.g. CLL), or when cytogenetic culture for karyotype analysis fails, in order to confirm or exclude specific cytogenetic abnormalities e.g. *BCR::ABL1* rearrangement in CML. Cytogenetic remission of previous abnormalities post therapy is assessed by FISH, and it is used to confirm relapse of disease.

FISH can be performed on metaphase or interphase cells on cultured blood or bone marrow, and on interphase cells from blood and bone marrow smears, cytospin preparations from CSF or pleural effusions and tissue sections.

Specimens required

Karyotyping:
Bone marrow aspirate (1–2 mL) in bone marrow transport medium containing RPMI medium, heparin and antibiotics (available from the laboratory on request). If unavailable, samples should be placed in lithium heparin.

FISH:
Ideally performed on EDTA. In certain instances, FISH may be performed on formalin fixed, paraffin embedded tissue sections. Please call the laboratory to arrange for these to be done, and for further details on sample requirements.

SNP arrays:
EDTA is preferred, although bone marrow transport medium may also be used. DNA obtained from formalin fixed material is not suitable for arrays.

Reporting

The cytogenetics report will provide the karyotype of the cell population analysed and/or abnormalities detected by FISH. An overall conclusion and interpretation (including prognostic significance where known) will be given in the primary report and this will be interpreted in light of the other findings in the integrated report.

Molecular Genetics

Scientists in Charge:	Dr Ilenia Simeoni (myeloid)	(01223) 348866
	Calogero Casa (lymphoid)	(01223) 348866

The East GLH molecular genetics laboratory works in partnership with HODS to offer a wide range of DNA- and RNA-based molecular tests to identify acquired genetic changes of diagnostic, prognostic and therapeutic significance in both haematological and solid tumour malignancies.

Please refer to the NHS England National Genomic Test Directories for a comprehensive list of haemato-oncology molecular assays, not all of which are provided by each GLH. Please see also the East GLH website: https://www.eastgenomics.nhs.uk/. The GLH results are integrated with other testing modalities provided by HODS. Enquires about sample requirements, turn-around-times and availability of results can be sent to cuh.eastglh-haemonc-molecular@nhs.net.

Many haematological malignancies have characteristic genetic abnormalities that are either the key entity-defining criteria or important for diagnosis and management. Some abnormalities occur at the chromosomal level and lead to the production of a novel fusion gene or aberrant expression of an oncogene. These abnormalities can be detected by cytogenetic methods and confirmed by molecular methods. In addition, an increasing number of subtle mutations are associated with malignancies and these are not detectable by cytogenetic methods.

Available methods include:

- Polymerase Chain Reaction (PCR)
- Reverse Transcription PCR (RT-PCR)
- Real Time PCR
- Sanger Sequencing
- High-Resolution Melt Analysis
- Fragment Analysis
- Pyrosequencing
- Next-Generation Sequencing (NGS)

Molecular genetic analysis is often the most sensitive technique for detecting minimal residual disease (MRD). Other assays use short tandem repeats (STR) to assess the proportion of cells carrying donor-derived haematopoietic cells after an allogeneic stem cell transplant (e.g. for chimerism analysis).

Next generation sequencing (NGS)

Next generation sequencing (NGS) is an accurate, high-throughout, and cost-effective alternative to single-gene methods and can simultaneously identify mutations across multiple genes in one assay from one sample. The GLH laboratory currently offers several NGS-based assays, with panel testing expected to replace many single-gene tests.

Targeted NGS panels do not always examine the entire gene, so absence of a variant does not completely rule out the presence of a mutation elsewhere in the gene. The formal lower limit of detection is 5%, although lower-level mutations will typically be reported if confidently detected.

Variants of uncertain significance

Some gene variants may be novel and will be reported as 'variants of uncertain significance', which can be discussed with a HODS consultant if the clinical implications are unclear. Formal Genomic Tumour Advisory Boards meet weekly to discuss the significance of challenging variants.

Germline variants

NGS panels on a diagnostic haematopathology sample sequence a tumour sample and are not directly intended to identify germline variants. However, reports will state if any potential germline variants have been detected (e.g. variant allele frequencies > 30%). Where potential pathogenic variants have been identified in genes pertinent to germline predispositions to haematological malignancies, cases will routinely be referred to the East GLH Genomics Tumour Advisory Board so that appropriate advice can be given about further counselling and testing.

Tests performed by the East GLH

Liquid samples:
A separate peripheral blood (PB) or bone marrow (BM) 3–5 mL EDTA sample is required (20 mL PB for *BCR::ABL1* quantification and post-transplant chimerism analysis).

To ensure RNA integrity, samples should be received within 24 hours of collection, and arrive before 15:00 on Friday. Aged samples (>48 hours) may be rejected as tests may not be reliable in such cases (especially if *BCR::ABL1* quantification is requested).

Formalin-fixed, paraffin-embedded (FFPE):
FFPE-fixed material can be used for NGS (e.g. for B- and T-cell clonality assessment) when the disease burden in liquid samples is insufficient.

FFPE-fixed material is not suitable for most RNA-based standalone tests. RNA fusion panels are available at laboratories external to the East GLH.

Tests not performed by the East GLH

If these are the only tests required (e.g. *BCR::ABL1* TKD mutation testing on PB, AML MRD on PB), they can be sent directly to the appropriate GLH by the referring hospital/clinician. Samples are dispatched unprocessed, whenever possible, to avoid delays and improve result quality.

Acute leukaemia MRD samples:
See the relevant section in "How to submit samples"

Reporting

A molecular diagnostic report will be provided with an interpretive comment on the findings.

Tests performed by an external laboratory will be reported by the external laboratory. These results will also be included in the SIHMDS integrated report for bone marrow samples.

Complaints, concerns and compliments

HODS is always keen to improve our service. Complaints or concerns can be raised initially with the Clinical Lead or the Operational Lead, although if they are not available any member of the HODS staff can also be contacted. Confirmation of receipt, if required, will be given within 2 working days with a formal response in 10 working days. If such attempts to resolve the issue are not successful, you can also contact the PALS team (Patient Advice & Liaison Service; telephone 01223 216756 or email cuh.pals@nhs.net).

Patients who wish to provide feedback on the service are also encouraged to contact PALS.

We are also happy to receive compliments about good service and will ensure these are shared with the relevant staff members.

Further information about the departmental complaints, concerns and compliments policy is available on request. Additional Trust information is available here:

https://www.cuh.nhs.uk/contact-us/let-us-know-your-views/

Data Protection and GDPR

The laboratory adheres to the Trust's Policy on the strict management of Information Governance to comply with the Data Protection Act and the General Data Protection EU Directive (GDPR) when handling patient data.

All staff within the laboratory complete mandatory Information Governance and GDPR training and are aware of the requirements to maintain confidentiality and the restrictions imposed on the laboratory with regard to information dissemination. If you have any further query please contact the laboratory or email cuh.gdpr@nhs.net

Appendix 1: HODS Request Form

The HODS Request Form is available online as a fillable PDF form and can be downloaded using the link below. Alternatively, you may print the form on the following page of this handbook.

DOWNLOAD REQUEST FORM

PATIENT LABEL

Cambridge University Hospitals
NHS Foundation Trust

HAEMATOPATHOLOGY AND ONCOLOGY DIAGNOSTIC SERVICE REQUEST FORM

ADDRESS	BOX 234, ADDENBROOKE'S HOSPITAL, HILLS ROAD, CAMBRIDGE, CB2 0QQ
PHONE	(01223) 217132 OR EXTENSION 217132
EMAIL	add-tr.hods@nhs.net (OR SEARCH 'HODS' ON NHS.NET DIRECTORY)

ALWAYS NOTIFY URGENT REQUESTS AND COMPLETE THE REFERRAL INFORMATION

PATIENT DETAILS		REFERRAL INFORMATION	
SURNAME		CLINICIAN	
FORENAME(S)		HOSPITAL	
DATE OF BIRTH	M ☐ F ☐	WARD/CLINIC	
HOSPITAL NO.		PHONE/BLEEP	
NHS NO.		EMAIL (NHS.NET)*	
CATEGORY	☐ NHS ☐ PRIVATE	INVOICE DETAILS (IF DIFFERENT TO REFERRER)	
		PHONE/EMAIL*	

CLINICAL DETAILS

RECENT FBC	DATE:				DANGER OF INFECTION?	☐ Y	☐ N
WBC		x10⁹/L	HB	g/L	SUSPECTED / KNOWN TB?	☐ Y	☐ N
NEUTROPHILS		x10⁹/L	MCV	fL			
MONOCYTES		x10⁹/L	PLATELETS	x10⁹/L	AFFIX DOI STICKER		
LYMPHOCYTES		x10⁹/L	OTHER				

SAMPLES ENCLOSED:	TESTS REQUESTED:
☐ PERIPHERAL BLOOD	☐ ACUTE LEUKAEMIA MOLECULAR MINIMAL RESIDUAL DISEASE†
☐ BONE MARROW ASPIRATE	☐ MORPHOLOGY
☐ BONE MARROW TREPHINE	☐ FLOW CYTOMETRY
☐ CSF	☐ FISH
☐ TISSUE BIOPSY, SPECIFY:	☐ KARYOTYPING
☐ OTHER, SPECIFY:	☐ MOLECULAR
	☐ OTHER, SPECIFY:

SPECIMEN(S) COLLECTED BY:		CONTACT DETAILS:	
PRINT		ROLE	
SIGNED		PHONE/BLEEP	
DATE & TIME		EMAIL (NHS.NET)*	

SAMPLE RECEIPT (FOR INTERNAL USE):

DATE & TIME		INITIALS:			
☐ BLOOD	☐ BM ASPIRATE	☐ BM TREPHINE	☐ CSF	☐ SLIDES	☐ OTHER

SAMPLE DESCRIPTION, INCLUDING TISSUE DIMENSIONS (MM):

*Submitter's email address will be used for direct queries from the HODS laboratory staff to the referring clinician. Reports will be routed to the referring hospital's Net Delivery Address registered with the laboratory.

†Acute leukaemia molecular MRD samples should preferably be received in the lab before 3 pm Mon to Thurs. Please phone to inform lab of samples arriving after this time.

Appendix 2:
Instructions for couriers delivering samples out of hours for HODS.

Couriers should come to the Haematopathology and Oncology Diagnostic Service (HODS) department which is located on level 3 in the pathology block. The entrance to HODS is swipe access only so the courier should contact the blood transfusion department on x596263. The phone and phone number is located to the left of the entrance door.

If the courier is arriving via the Pathology entrance door this access is restricted by swipe card between the hours of 17.00 – 07.00. The courier will have to go to the main entrance and ask the reception staff to call blood transfusion.

A blood transfusion staff member will sign for the samples and place these samples in the "HODS Out of Hours fridge".

Appendix 3: CUH bone marrow aspirate sampling guidance (including MRD sample requirements)

This suggests the appropriate samples to take depending on the possible diagnosis at the time of bone marrow sampling. Please also see bone marrow sampling procedure for technical aspects of taking a bone marrow sample. For controlled document please see Connect website on CUH Intranet**.**

At diagnosis/relapse of acute leukaemia or pancytopenia of unknown cause

Taken in order (please note both blue and red tubes in CUH contain EDTA):

- Bedside slides for morphology.
- 2-3 mL in EDTA for flow cytometry (1 x red).
- 2 x 3–5 mL in EDTA tube for molecular testing (2 x blue tubes) – one for in-house testing, one for MRD marker detection if ALL.
- 2-3 mL in EDTA for FISH (suggest blue).
- 2–5 mL in cytogenetics media for karyotyping.
- Trephine biopsy recommended in all cases unless known / likely APML.

Notes:
The results of FISH and FLT3/NPM1 testing are important when choosing initial treatment. For expediency, please send separate samples for each test to avoid delays from sharing samples between laboratories.

In the event of a "dry tap", a second trephine biopsy should be collected into a universal container (in 1 mL of sterile saline) for disaggregation in order to perform the above tests. In addition, if circulating blasts, please send 20 mL of PB in EDTA for testing and an additional 10 mL in a cytopot. Discuss with the HODS team.

AML follow up post therapy (including MRD)

If no clinical suspicion of relapse (blood counts stable and asymptomatic, and no suspicion of graft failure if post allograft):

- Bedside slides for morphology – < 0.5 mL, 2-3 slides sufficient, put any excess into first pull tube.

If molecular marker detected (this is documented on alert section of EPIC and in the diagnostic sample integrated report):

-2-3 mL in EDTA for molecular MRD assessment – labelled "first pull".

- 2-3mL in EDTA for flow cytometry (second pull – will only be needed if increased blasts by morphology).

If no molecular marker detected:

-2-3 mL in EDTA for flow cytometry – labelled "first pull".
– FISH can be performed on this sample if relevant.

- Trephine biopsy if applicable (see notes below).

Notes:

Molecular MRD

All samples for molecular MRD testing that are received via HODS must state "FOR MOLECULAR MRD" on the request form.

If NPM1 mutated AML, post cycle 2 of DA-based chemotherapy please send 20 mL PB for NPM1 quantification and note this in the clinical details.

MRD tests that require RNA extraction include (but are not limited to) NPM1, BCR::ABL1 (p190), PML::RARA, CBFB::MYH11 and RUNX1::RUNX1T1. These can usually be dispatched to external laboratories via the GLH without processing if they are received on Monday-Thursday, either on the same day as collection or if the collection date was the day prior and the sample is received before 1.30pm. Samples not received in this timeframe will require processing in the GLH prior to external dispatch and overall turnaround times will be delayed.

Trephine biopsy

If good/particulate aspirate obtained, trephine biopsy is not needed post-chemotherapy. If insufficient aspirate obtained, review the pre-treatment blast phenotype. It is often very difficult to enumerate CD34-negative blasts on a trephine biopsy, so retry for a good aspirate sample. Please seek guidance from HODS team member or clinical consultant if uncertain of sample requirements.

Trephine biopsy should be included as part of the "Day +100" post-transplant assessment; however, it is not required with every BM biopsy in the post-transplant setting provided that a good/particulate aspirate sample has been obtained, blood counts are stable, and there are no clinical concerns of graft failure.

ALL follow up post therapy (including MRD)

Post phase 1, and subsequent cycles (note: post phase 1 is a decision point for UKALL2011 protocol, and post course 2 is a decision point for UKALL14 protocol, but sample requirements are the same):

- Bedside slides for morphology
- 2-3 mL in EDTA for IgH /TCR molecular MRD assessment (label first pull).
- 2-3 mL in EDTA for flow cytometry.
- Additional 2-3 mL in EDTA for *BCR-ABL1* for Ph+ ALL.
- Trephine biopsy if applicable (see notes below).

Notes:

Molecular MRD

All samples for molecular MRD testing that are received via HODS must state "FOR MOLECULAR MRD" on the request form.

MRD tests that require RNA extraction include (but are not limited to) BCR::ABL1 (p190). These can usually be dispatched to external laboratories via the GLH without processing if they are received on Monday-Thursday, either on the same day as collection or if the collection date was the day prior and the sample is received before 1.30pm. Samples not received in this timeframe will require processing in the GLH prior to external dispatch and overall turnaround times will be delayed.

Trephine biopsy

A trephine biopsy is usually not needed for non-transplanted ALL follow up.

Trephine biopsy should be included as part of the "Day +100" post-transplant assessment; however, it is not required with every BM biopsy in the post-transplant setting provided that a good/particulate aspirate sample has been obtained, blood counts are stable, and there are no clinical concerns of graft failure.

Myeloma or lymphoma

- Bedside slides for morphology

- 2-3 mL red EDTA for flow cytometry
- 2-3 mL red EDTA for FISH (NOT IN CYTOPOT – CYTOPOT NOT NEEDED unless there are unexplained cytopenias ?MDS)
- Trephine biopsy (important as myeloma is a patchy disease)

Please note that at diagnosis FISH is increasingly useful. Therefore if the aspirate is aparticulate it is worth re-attempting the aspirate to try and obtain improved samples which will increase the chance of obtaining a result.

MPN (including CML) and MDS or unexplained cytopenia

- Bedside slides for morphology
- 2-3 mL EDTA for flow cytometry
- 2-3 mL in EDTA for FISH if needed
- 2-3 mL EDTA for molecular testing
- 5–10 mL in cytogenetics media for karyotyping
- Trephine biopsy (often the most diagnostic test so important to obtain an adequate sample) – BUT no trephine biopsy needed in CML if good particulate aspirate obtained

Sample requirements summary

Disease	New/relapse acute leukaemia, or unexplained cytopenia	AML follow up (if no suspicion of overt disease)	ALL follow up	Myeloma And lymphoma	MPN/CML and MDS
Bedside slides #	4-5	3	3	3	4-5
Flow (EDTA)	Y	Y First pull if NO molecular marker	Y First pull if NO molecular marker	Y	Y
Molecular testing including MRD and chimerism (EDTA)	Y (2 tubes)	Y First pull if molecular marker	Y First pull if molecular marker (plus extra tube if Ph+)	Y	Y
FISH (EDTA)	Y	Y	N	Y	Y
Karyotype (cytopot)	Y	N	N	N (unless unexplained cytopenia)	Y
Trephine	Y (unless known / suspected APML)	Not usually needed– see notes	Not usually needed, see notes	Important	Important in MPN, helpful in MDS, not usually needed in CML (see notes)
Total EDTAs	**4**	**2-3**	**2-3**	**3**	**3**

Appendix 4: Ordering samples on EPIC within CUH

Staff working within CUH can request HODS investigations on Epic. The most common orders are listed below, along with suggestions regarding their use:

1. Suspected Haematological Malignancy Panel (SHMP)
2. Known Haematological Malignancy Panel (KHMP)
3. CSF (Haematology)
4. Chimerism and specific genomic tests on peripheral blood

Suspected Haematological Malignancy Panel (SHMP)

To submit specimens (e.g. peripheral blood, bone marrow, or other body tissues or fluids) for diagnostic investigation of a patient with a suspected haematological malignancy, order a "Suspected Haematological Malignancy Panel" on Epic and select the required test(s) from the test list (as detailed below):

- Peripheral Blood Collection for Flow and Morphology
- Peripheral Blood Collection for FISH
- Peripheral Blood Collection for Karyotype
- Peripheral Blood Collection for Molecular Genetics
- Bone Marrow Aspirate Collection for Morphology, Flow and FISH
- Bone Marrow Aspirate Collection for Karyotype
- Bone Marrow Aspirate Collection for Molecular Genetics
- Tissue Collection for Morphology
- Tissue Collection for Flow
- CSF Collection
- Other Body Fluid Collection (please specify)
- BM Trephine Collection

Notes:

All bone marrow biopsy investigations should be accompanied by a contemporaneous peripheral blood sample, and a request placed for "Peripheral Blood Collection for Flow and Morphology" from the SHMP test list.

Known Haematological Malignancy Panel (KHMP)

To submit specimens (e.g. peripheral blood, bone marrow, or other body tissues or fluids) for disease assessment of a known haematological malignancy, order a "Known Haematological Malignancy Panel" on Epic and select the required test(s) from the test list (as detailed for the SHMP).

CSF (Haematology)

When submitting CSF specimens for investigation of a suspected or known haematological malignancy (e.g. by cytospin ± flow cytometry), do not use the option "CSF Collection" from the SHMP test list. Instead, it is preferable to request "CSF (Haematology)" from the Order menu.

Chimerism and specific genomic tests on peripheral blood

Please see the following link for information on Connect about ordering these tests in EPIC:

https://www.cuhconnect.nhs.uk/our-structure/a-z-teams/genomics/ordering-genomics-tests-in-epic/

Appendix 5: Antibody panels in current use

Panel	Identification of cell types
Acute leukaemia panel	*T-cells:* CD1a, CD2, CD3 (surface and cytoplasmic), CD4, CD5, CD7, CD8, CD99
	B-cells: CD9, CD19, CD20, CD22, CD79a(cy)
	Myeloid: CD11b, CD13, CD14, CD15, CD16, CD33, CD64, CD117, myeloperoxidase (MPO)
	Blasts: CD34, TdT
	Others: CD10, CD36, CD45, CD56, CD61, CD123, CD303, CD304, HLA-DR
Lymphocyte panel	*T-cells:* CD2, CD3, CD4, CD5, CD7, CD8, PD1, CD30
	B-cells: CD19, CD20, CD22, CD23, CD79b, CD103, Kappa/Lambda light chains
	NK-cells: CD2, CD16, CD56, CD57
	Others: CD10, CD11c, CD25, CD38, CD43, CD45, CD81 CD123, CD200, PD1
Plasma cell panel	Cytoplasmic Kappa/Lambda, CD19, CD20, CD38, CD45, CD56, CD138
Stem cell enumeration	CD34, CD45
PNH	*GPI-linked antigens:*
	CD24, CD66b (neutrophils)
	CD14 (monocytes)
	CD59 (red cells)
	Fluorescent Aerolysin (FLAER), as required
Hereditary Spherocytosis screening	Eosin-5-maleimide
Immunological platelet enumeration	CD61 / CD41 ICSH reference method

Based on the initial results, other antibody combinations may be used. For more information on monoclonal antibody specificity please go to: https://www.pathologyoutlines.com/cdmarkers.html