Unique Identifier	MI-CG-CPP-User Guide	Version No.	6.6	The Christie Pathology Partnership A joint venture with SYNLAB
Approval	C. Hodgson, M. Green, K. Stanley	Date of Issue	03/04/2023	
Author	N. Telford	Frequency	Biennial	

Oncology Cytogenetics User Guide 2023

Contents

Introduction

Oncology Cytogenetics of The Christie Pathology Partnership is a specialist regional service, which provides genetic testing to aid diagnosis of leukaemia and other tumours, to hospitals in Greater Manchester and the North West of England. Malignant diseases are often classified by their genetic abnormalities and certain cytogenetic tests are essential for the optimal diagnosis and treatment stratification of cancer patients. Increasingly, cancer drugs are being developed that target specific genetic lesions, which therefore require a predictive molecular test.

The Oncology Cytogenetics service is a section of the pathology department operated by The Christie Pathology Partnership, which is a limited liability partnership between The Christie NHS Foundation Trust and Synlab UK. The Oncology Cytogenetics laboratory is accredited by UKAS to ISO15189:2012 standards (ref. no. 8697). The laboratory also complies with professional standards issued by the Association for Clinical Genomic Science (ACGS) and participates in all relevant EQA schemes (GENQA and UKNEQAS). The comprehensive cytogenetics service is provided by a highly-skilled team of dedicated scientists and technologists who offer a timely, efficient and cost-effective analytical and genetic advisory service to clinicians, to aid the diagnosis and monitoring of leukaemia and solid tumours. All relevant staff are state registered and clinical scientists are formally trained in Clinical Cytogenetics and Molecular Cytogenetics. We are a founder member of the UK Cancer Cytogenetics Group (UKCCG).

Handling more than 5,000 referrals per year, we are one of the largest specialist cancer genetics units in the UK. The department is the nominated service for leukaemia cytogenetics testing for Greater Manchester Cancer and provides services as part of the Manchester Haematological Cancers Diagnostic Partnership (HCDP).

http://haematologyetc.co.uk/Manchester_Haematological_Cancers_Diagnostic_Partnership

Oncology Cytogenetics provides cytogenetics and FISH services for the Lancashire & South Cumbria Haematology Network, the samples of which are also tested at the Haematological Malignancy Diagnostics Service (HMDS) in Leeds. We enter our results and upload a copy of the report on corresponding samples to the HMDS database, to inform the integrated report and the final diagnosis (see Reporting to HMDS Leeds below).

The laboratory is part of the National Genomic Laboratory service and is a Local Genomics Laboratory (LGL) in the network of laboratories led by the NW Genomics Laboratory Hub at Manchester Foundation Trust (https://mft.nhs.uk/saint-marys/services/genomic-medicine/north-west-genomic-nw-glh/). The services described in this document are prescribed in the National Genomic Test Directory, which underpins the genomic laboratory network.

Working closely with the specialist Histopathology and Breast Tumour Receptor sections of Christie Pathology Partnership, we provide FISH testing for the diagnosis of various solid tumours, including regional services for HER2 testing in breast and oesophageal carcinoma. We are continually developing assays to expand the diagnostic FISH service and provide specialised testing for clinical trials.

Contact Details

Address

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The Christie Pathology Partnership
The Christie NHS Foundation Trust
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Withington
Manchester M20 4BX

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Main Departmental Contacts

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Hours of Operation

Monday to Friday	8.30am to 5pm
Weekends	There is no routine service at weekends. Samples requiring special attention should be arranged in advance.
Bank Holidays	The department is not routinely staffed on Bank Holidays. For urgent attention, contact The Christie switchboard (0161 446 3000). A letter is sent to regular service users, in advance, detailing arrangements at Christmas and Easter.

Samples

Sample Types

Bone Marrow is the tissue of choice to investigate patients suspected of having leukaemia or related haematological neoplasms. Bone marrow aspirate specimens are routinely received although a bone marrow trephine specimen is an option if the marrow is fibrotic or otherwise difficult to aspirate.

Peripheral Blood can be sent if disease cells are present in sufficient numbers to allow cell culture and/or FISH studies, as appropriate. Blood is satisfactory for FISH studies in CLL if there is peripheral blood lymphocytosis. Peripheral blood is acceptable in plasma cell leukaemia but not myeloma.

Other fresh tissues can be analysed and lymph nodes, spleen, ascitic fluid, CSF and solid tumours are occasionally received. We do not have facilities for long-term culture for a comprehensive solid tumour karyotyping service, although we are pleased to accept fresh solid tissue to make touch imprints if FISH testing is indicated.

Paraffin Embedded Tissue for FISH on solid tissue, such as lymphoma, breast, sarcoma or brain tumour patients. Please send 3~4 µm tissue sections (see Fluorescence in situ Hybridisation (FISH)).

Specimen Containers for Fresh Specimens

The laboratory will provide containers to regular referrers for bone marrow and blood collection. These sterile bottles contain heparinised tissue culture medium with antibiotics, to facilitate the transport of the small amount of bone marrow and avoid desiccation. The COSHH assessment (available on request) of the contents shows that they are not toxic or otherwise hazardous but could be a mild irritant and should be handled with caution using protective gloves.

An allocation of specimen bottles will be issued at the beginning of each week/month based on the number of samples usually received. More bottles can be sent upon request, at any time, by hospital transport or by post. The bottles are given a two month expiry date; please verify that the bottle is in date prior to use and that the liquid normal pink and not discoloured. In emergency, a blood tube containing lithium heparin can be used.

Use only heparinised containers.
Please DO NOT use other anticoagulants such as EDTA, which is toxic to cells.

Collection of bone marrow aspirate and blood samples and the disposal of materials used in collection should be carried out according to local trust protocols. Fresh solid tissues should be placed in one of our Transport Bottles or other sterile liquid such as culture medium, Hank's balanced salt solution or saline.

The department is pleased to advise on the use of alternative specimen containers.

Dispatch of Fresh Samples

All sample bottles should be fully labelled and placed in a plastic specimen bag with request card in separate pocket. Samples should be packed in sufficient absorbent packing material to soak up the entire contents in the event of leakage, and placed in a cardboard sample box or other recommended receptacle.

Samples sent through the post, taxi or other courier service should comply with Packaging Instruction 650 and regulation UN3373.
Any packaging should bear the UN3373 diamond mark and labelled "Biological Substance, Category B" in letters at least 6mm high (e.g. see below).

BIOLOGICAL SUBSTANCE, CATEGORY B

Fresh samples should be sent to the laboratory as soon as possible, preferably on the day of collection, but should be received within 24 hours. Samples not being sent immediately should be refrigerated overnight at 4^{o}C and sent at the earliest opportunity the following day. **Please keep samples with suspected myeloma at room temperature; refrigeration is thought to impair selection of CD138+ cells.**

First class post is usually acceptable. At times, it may be necessary to send specimens to the laboratory by taxi or courier, to avoid delays, especially approaching weekends and bank holidays.

It is advisable that all Friday samples arrive on the day of collection to ensure that they are set up in culture before the weekend. Myeloma samples need to arrive before 3pm on Fridays to allow time for cell separation. **We cannot routinely receive High Risk samples on a Friday (see Policy for High Risk Samples).**

Please send samples at the earliest opportunity.
It is advisable to telephone about any samples that could arrive at the laboratory late in the day or out of hours.
The Duty Scientist may advise sending the sample the following day.

Request Cards

Please fill in all the patient demographics on the request card.

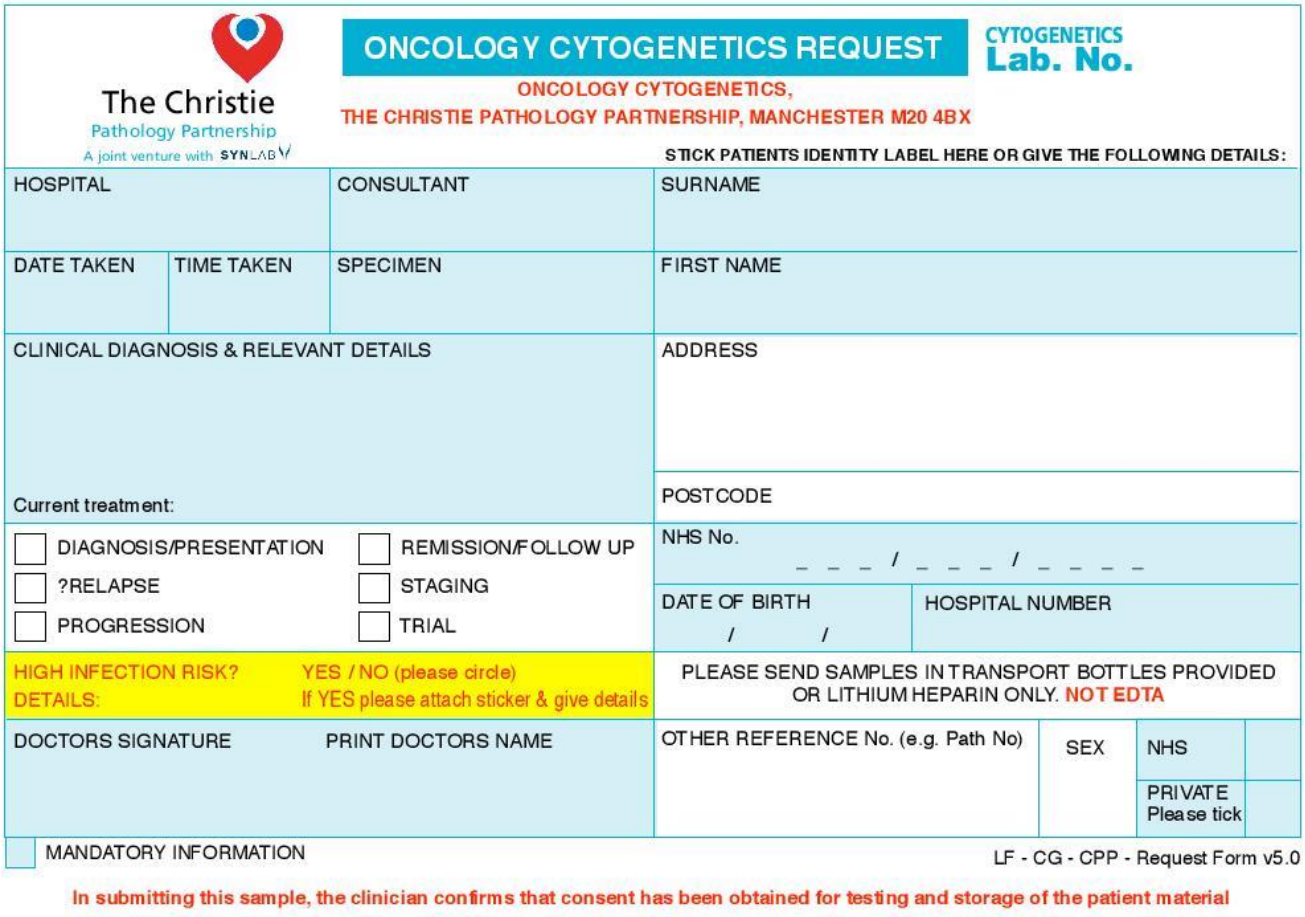
The reason for referral is important to determine which culture types need to be set up, which tests to perform, numbers of cells to analyse and sample prioritisation. All relevant clinical and haematological information and likely diagnosis can be included. If the patient is a participant of a **research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

The department operates a Specimen Acceptance Policy [MI-Pathgen-Christie-specimenacceptance]. The following details are essential requirements for request cards and specimens:

Request Card	Specimen bottle
1. Patients full name and date of birth. 2. Hospital number and NHS number. 3. Laboratory, Histology or HMDS lab nos. (as appropriate) to coordinate with other lab investigations. 4. Reason for referral/clinical information. 5. Specimen type. 6. Consultant name or initials and hospital. 7. Requestor's name and signature. 8. Date specimen was taken. 9. Time specimen was taken. 10. High risk status (if appropriate) with high risk label. 11. Private patient (if appropriate).	1. Patients full name, with hospital number (or NHS number) and/or date of birth. 2. Specimen type and site of specimen to distinguish multiple specimens. 3. High-risk label (if appropriate) 4. Date specimen was taken 5. Time specimen was taken.

Please use the current version of the request card.
Please see following image of version 5.0

Request Form v5.0
(front)



The Christie
Pathology Partnership
A joint venture with SYNLAB

ONCOLOGY CYTOGENETICS REQUEST

CYTOGENETICS **Lab. No.**

ONCOLOGY CYTOGENETICS,
THE CHRISTIE PATHOLOGY PARTNERSHIP, MANCHESTER M20 4BX

STICK PATIENTS IDENTITY LABEL HERE OR GIVE THE FOLLOWING DETAILS:

HOSPITAL		CONSULTANT	SURNAME
DATE TAKEN	TIME TAKEN	SPECIMEN	FIRST NAME
CLINICAL DIAGNOSIS & RELEVANT DETAILS Current treatment:			ADDRESS POSTCODE
☐ DIAGNOSIS/PRESENTATION ☐ REMISSION/FOLLOW UP ☐ ?RELAPSE ☐ STAGING ☐ PROGRESSION ☐ TRIAL			NHS No. _ _ _ / _ _ _ / _ _ _ _ DATE OF BIRTH / / HOSPITAL NUMBER
HIGH INFECTION RISK? YES / NO (please circle) DETAILS: If YES please attach sticker & give details			PLEASE SEND SAMPLES IN TRANSPORT BOTTLES PROVIDED OR LITHIUM HEPARIN ONLY. NOT EDTA
DOCTORS SIGNATURE	PRINT DOCTORS NAME		OTHER REFERENCE No. (e.g. Path No) / SEX / NHS / PRIVATE Please tick

☐ MANDATORY INFORMATION

LF - CG - CPP - Request Form v5.0

In submitting this sample, the clinician confirms that consent has been obtained for testing and storage of the patient material

All blue shaded areas are considered mandatory and should be completed before sending the form

Request Form v5.0 (reverse)

- **Hours of Operation:** Monday to - Friday 8.30am to 5.00pm. There is no routine service at weekends or bank holidays.
- **HIGH RISK samples should be arranged in advance.**
- Please use Cytogenetics Transport Bottles, supplied to regular referrers, for bone marrow and blood collection.
  In an emergency, a blood tube containing lithium heparin can be used
  Please DO NOT use other anticoagulants such as EDTA.
- Samples should be sent to the laboratory as soon as possible, within 24 hours.
  Samples not for immediate dispatch should be refrigerated.
- **All Friday samples need to arrive by 4pm on the day of collection. For Myeloma samples 3pm to allow for cell separation.**
- **It is necessary to telephone about any samples that could arrive to the laboratory out of hours.**
- **Enquiries:** 0161 446 3165/8608
- **For full details see website for Oncology Cytogenetics User Guide**
  https://www.christie.nhs.uk/services/i-to-q/pathology/oncology-cytogenetics/

FOR LABORATORY USE ONLY

DATE REC	TIME REC	CULTURES REQUESTED	PRIORITY	DUTY SCIENTIST

VOLUME	CELL COUNT	CULTURES SET UP	SET UP BY	TESTS REQUIRED
				☐ CYTO ☐ CYTO FISH ☐ FISH ONLY ☐ PET FISH

CD138 date set up:	C culture/s set up by:	TRANSPORT BOTTLE EXPIRY DATE / /
Set up by:	Pour off volume:	COMMENTS
CD138 volume:	Cell count:	
	Volume per culture:	

AP1369

Policy for High Risk Samples

All samples from patients exposed to a dangerous infectious pathogen (ACDP category 3 or higher, including HIV and Hepatitis B and C) will be considered a high infection risk. This includes known carriers and patients with prior exposure to people infected with 3+ dangerous infectious pathogens.

All samples from patients at High Risk of infection referred for cytogenetic analysis should be identified to the laboratory. The sample and request card must be clearly labelled as High Risk. It is advisable that patients at risk of infection are discussed in advance.

HIV, Hepatitis B or Hepatitis C samples can be processed, if cytogenetic analysis is critical to patient management.

All other samples at High Risk of infection (with ACDP category ≥3 pathogen) cannot be processed by the laboratory.

Samples at risk of infection with HIV, Hepatitis B or Hepatitis C, may be processed by the laboratory. However, as the samples require special attention, we request that these are arranged in advanced between the Oncology Cytogenetics laboratory and the referring clinician. In particular, High Risk samples cannot be processed over a weekend and samples can only be accepted on a Friday in exceptional circumstances. Special arrangements must be made for Friday samples. Full cytogenetic analysis will only be considered in circumstances where a result will directly influence patient management.

The culture of cells from HIV, Hepatitis B or Hepatitis C samples requires special attention in isolation conditions. Processing of these samples will therefore incur an additional charge to the referring department. Alternatively, uncultured specimens can be fixed for FISH only but a full cytogenetic result will not be possible.

Any samples at risk of infection with any other ACDP category 3 pathogen (or higher) will not be processed by the laboratory.

Any sample of uncertain risk status of infection with ACDP category 3 pathogen or if satisfactory arrangements cannot be made, will be disposed of by incineration.

HIV, Hepatitis B and Hepatitis C samples received where no arrangements can be made or without strong indication for cytogenetic analysis, will be fixed uncultured for possible FISH only. Consequently, a conventional cytogenetics result will not be possible.

In all instances, a record of the actions taken will be made and a report issued to the referring consultant.

*Revised Advice on Laboratory Containment Measures for work with Tissue Samples in Clinical Cytogenetics Laboratories [2001]

Fresh samples with novel coronavirus (SARS-CoV-2).

According to current advice regarding laboratory handling and processing of samples with SARS-CoV-2, from Gov.UK (https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/wuhan-novel-coronavirus-handling-and-processing-of-laboratory-specimens) SARS-CoV-2 is considered a Hazard Group 3 level pathogen according to a provisional classification by the ACDP committee.

Therefore, this is covered by this policy and samples from patients known to have SARS-CoV-2 from patients with COVID-19 infection cannot be set up in culture, to avoid inadvertent propagation of the virus and increased risk of infection. Conventional cytogenetic analysis will therefore not be possible.
We requested that fresh samples at risk of infection are not sent to the laboratory whilst the patient is at high risk of infection with COVID-19. Non-urgent cases can be investigated at a later date, when the patient has recovered from infection or test negative for the virus.

The Government guidance and PHE guidance; Health & Safety Guidance: Working with Samples suspected of containing high hazard agents (PHE document code HS002G v1.0) describes the conditions under which COVID-19 samples can be processed without culture. FISH testing for specific gene rearrangements is possible on uncultured cells. Samples which have to be sent during the course of infection will be fixed uncultured for possible FISH testing and full chromosome analysis would not be available.

Fixed material for FISH (e.g. FFPE tissue sections) are not affected by this policy.

Unlabelled High Risk Specimens

Receipt of unlabelled High Risk specimens is a recurring problem in the laboratory. It is important to correctly label High Risk samples as they are processed by different protocols and it may not be possible to culture some samples due to the risk of propagating a pathogen.
This policy for the acceptance of High Risk samples and for sample labelling is described above (page 11).

Unlabelled High Risk samples will be;

- Reported as an Incident to Datix (if a Christie sample)
- Reported to the referring consultant (using High Risk Letter to Clinicians template [LF-CG-CPP-HighRiskLetter] on QPulse.
- Trends are reported to the Quality Manager of the referring institution
- The sample will not be processed if there is any uncertainty in its status
- An additional handling fee will be charged

Policy of Consent for Testing and Retention of Samples

In submitting a sample to Oncology Cytogenetics, the clinician confirms that consent has been obtained for testing and storage of the patient material. Samples are tested and fixed surplus cells are retained for possible Oncology Cytogenetics use only, in connection with the original reason for referral. It will not be passed on to other parties or used for research or purposes other than the reason that they were originally referred. Samples may be retested for internal quality control, verification and validation of methods or for clinical audit, as required.

The Oncology Cytogenetics department currently retains fixed cell suspensions from samples for 8 years. This allows for further testing of samples and is particularly useful for additional FISH tests or when testing a diagnostic sample is required to establish a FISH signal pattern to enable testing of subsequent post-treatment samples. Used microscope slides from routine cytogenetic analysis are retained for 8 years and FISH slides for 1 year. Samples and slides are disposed of every 3 months following the completion of a full 8 years of age.

A sample not analysed at the time of referral can be reactivated at any time, if required.

Policy on Protection of Personal Information

The Oncology Cytogenetics department is required to process patients' information and produce sensitive diagnostic results as part of its routine service to support their clinical management. We ensure that all data is used and stored in strict accordance with Information Governance standards and the department is committed to meeting its information security obligations to fulfil the needs of users, clients, patients and staff with respect to confidentiality, integrity, and availability. All of the department's procedures and policies strictly comply with The Christie NHS Foundation Trust's polices:

- Data Protection Policy
- Freedom of Information Act Policy
- Information Governance Training Policy
- Information Governance & Security Policy

Laboratory Information Management is described in the **Pathology Quality Manual** (QI-PathGen-CPP-QualManual). Christie Pathology Partnership operates a **Security Policy** (SP-PathGen-Christie-Security) detailing physical security of the department. The management of data in Pathology IT systems and compliance with national legislation in relation to data protection is described in the **Pathology IT Policy** (MP-PathGen-Christie-IT). This includes provisions for data and network security, restriction of access, staff awareness of their duties in relation to the Caldicott report and the Data Protection Act and that breaches of security are investigated in the appropriate manner. Oncology Cytogenetics has IT specialists and local protocols for the operation of the laboratory database for input of records and transmission of data for reporting and clinical management. Contingency plans in the event of failure or downtime in the laboratory information management systems are described in the service continuity plan.

Sample Prioritisation and Reporting Times

Sample prioritisation and reporting performance targets of the professional standards of the Association for Clinical Genomic Science (ACGS) are used as minimum standards, including General Best Practice Guidelines, Haemato-Oncology Best Practice Guidelines and disease-specific Guidelines for CML/MPN, AML/MDS and ALL.

Urgent referrals (Acute leukaemia and CML at diagnosis or possible relapse)
All acute leukaemia and chronic myeloid leukaemia cases at diagnosis will be treated as 'urgent' and 95% of cases will be reported within 14 calendar days. In practice, the majority of these diagnostic cases are reported well within the internal reporting time target of 7 days. The first specimen after induction treatment in acute leukaemia is also treated as urgent.

Rapid FISH tests (e.g. APML, Burkitt lymphoma)
95% will be reported in 3 calendar days. In practice, the majority of cases will have a verbal report available within 24 hours or in 2-3 working days, if paraffin-embedded. A rapid FISH test for *PML-RARA* gene rearrangement in APML can be available within a few hours, if the sample is received by early afternoon.

Routine referrals for routine cytogenetic analysis
95% of all other referrals will be reported within 21 calendar days. The laboratory operates a 'priority' system whereby cases requiring quick attention or by special (telephone) request but which are not 'urgent' can be analysed out of turn.

In Abeyance
All samples that are not urgent and have an uncertain diagnosis will be held in abeyance, pending further information. Further details are requested on an interim report (by e-mail where available), which also permits you to suggest a priority level for the referral.

This is necessary because at the time of biopsy the diagnosis may not be known and chromosome analysis may not be required after bone marrow morphology is examined.
Consultants are requested to cooperate as fully as possible with this policy. This is to avoid unnecessary and labour intensive analytical work and helps the laboratory to process its large workload and minimise costs.

PET FISH
Reporting time targets for paraffin-embedded tissue sections referred for FISH have been agreed with local service users as follows;

HER2 on breast tumours	7 days for FISH (total turnaround including IHC = 10 days)
Lymphoma	7 days
Sarcoma	7 days
Brain tumours	14 days

Certain diagnoses and special requests can be turned around more rapidly (see above).
In the majority of cases PET FISH referrals are reported in a significantly shorter time.

Conventional Cytogenetic Analysis

Cytogenetic analysis is the process by which metaphase and interphase cells are analysed by light or fluorescence microscopy (with the use of image capture equipment as appropriate) to detect chromosome abnormalities, which can aid the diagnosis of leukaemia and other malignant conditions. These abnormalities can be numerical (loss or gain of chromosomes) or structural (e.g. translocations, inversions, deletions). Chromosome abnormalities can confirm a clonal disease and can often suggest a more specific diagnosis and prognosis. The abnormalities can be used to monitor remission and diagnose relapse, transformation or secondary disease. Increasingly, cytogenetic abnormalities indicate specific and targeted treatment regimes.

Cytogenetic techniques in bone marrow preparations

Conventional cytogenetic analysis relies on the culture of cells to produce metaphase chromosomes, where individual chromosomes can be visualised. Tissue, therefore, needs to be as fresh as possible with viable disease cells. Cells are processed and stained using ‘banding’ techniques to produce a karyotype. Abnormalities are defined and described according to the International System for Human Cytogenetic Nomenclature (ISCN).

Biological and clinical decision values

The examination of chromosomes can resolve genomic rearrangements down to a limit of approximately 5Mb of DNA, which depends on the morphology of the chromosomes of the neoplastic cells. Smaller genetic abnormalities may be present in disease cells which will not be detected by this technique. Statistically, a conventional 20 cell cytogenetic analysis will exclude an abnormal clone of 14% with 95% confidence (Hook, E B (1977) American Journal of Human Genetics 29(1): 94-97), although the performance of the technique for cancer cell detection will depend on the growth of neoplastic cells in culture which could improve sensitivity. This level of analysis is adequate for diagnostic samples but the sensitivity is limited for post-treatment monitoring or analysis of secondary tissue for staging. An abnormal clone is defined as two cells with the same structural chromosome abnormality or chromosomal gain or three cells with the same chromosomal loss. Additional cells are analysed in attempt to confirm single cell abnormalities as clonal, in accordance with Cytogenetics Analysis policy (LP-CG-CPP-Analysis).

An analysis that does not meet the minimum number of cells required will be qualified as a 'limited analysis' and a normal analysis with less than 10 cells available will be considered to have failed. If abnormalities commonly associated with the disease cannot be excluded and where clinical interpretation is compromised, the report will also be qualified. Caution will be exercised when interpreting results from fresh samples delayed in transit; any normal result from a fresh specimen that is more than 24 hours old will be qualified.

SNP Microarray Analysis

In 2023, Oncology Cytogenetics will launch a service for analysis of Single Nucleotide Polymorphism (SNP) profiling of haemato-oncology samples. The technology involves the hybridisation of test DNA to microarrays containing hundreds of thousands of unique nucleotide probe sequences. Each probe on the microarray is designed to bind to a target DNA sequence in the sample and specialised scanning equipment then measures the signal intensity of each probe after hybridisation with its target DNA. The signal intensity depends upon the amount of target DNA in the sample and a SNP genotype profile is constructed for contiguous regions of the genome of the specimen. SNP data can be used to detect copy number imbalances to 5Mb resolution (the sensitivity of conventional Cytogenetic Analysis), but also loss of heterozygosity (LOH) to 10Mb resolution, and more subtle copy number changes of key genes related to the reason for referral.

The SNP microarray service will supplement Cytogenetic analysis and FISH for the detection of abnormalities in haematological neoplastic disorders, in particular where a karyotype fails in MDS referrals and as an alternative to karyotype in ALL.

The service uses DNA samples processed by the Genomics Laboratory Hub at Manchester Foundation Trust and run on Illumina GSA Cyto+ SNP array (Global Screening Array-24+ v3.0). Data is analysed and interpreted (using NxClinicalTM software by Biodiscovery) and reports issued by Oncology Cytogenetics at CPP. An ETS will be requested and the service should be jointly accredited with the GLH in 2023.

Fluorescence in situ Hybridisation (FISH)

Fluorescence in situ hybridisation (FISH) uses fluorescently labelled gene probes to detect specific gene sequences on the microscope slide. This can be used to confirm specific genetic abnormalities at the molecular level. FISH can be used with metaphase chromosomes but is also applicable to interphase. Cell culture is not necessary and, therefore, FISH can be informative on fixed tissue such as FFPE. FISH can be used as a front-line test, if the reason for referral indicates, or as a secondary test to further characterise clinically relevant abnormalities following cytogenetic analysis. FISH is a targeted test which will detect rearrangements and copy number changes in the gene sequences tested for but not any other abnormalities in the genome.

FISH for haematological malignancies

FISH is applied to fixed cells from bone marrow and blood samples referred for investigation of possible haematological malignancy. An extensive range of FISH gene probes is available in the department to detect all common and many other recurrent abnormalities in haematological malignancy. The main FISH services for haematological malignancies are listed (Summary of Services Offered by Conventional Cytogenetics and FISH for Haematological Malignancies). However, it is not possible to detail all the tests available in this Guide. Please enquire if you require full details.

FISH on Paraffin-embedded Tissue (PET) for Solid Tumours

The FISH services offered on PETs for the diagnosis of solid tumours are listed (Summary of Services Offered by FISH on paraffin-embedded tissue for solid tumours) and further details about specific services in FISH on the section Paraffin-embedded Tissue for Solid Tumours.

Sample requirements for Paraffin-embedded Tissues

- The laboratory only accepts tissue sections. The optimal thickness for all sections is 3~4 µm. The laboratory currently does not accept uncut blocks of tissue, and these will be returned to the sender.
- Sections should be mounted on APES-coated (or equivalent) slides. Please label all slides clearly with AT LEAST THREE unique patient identifiers, e.g. name and pathology no.
- Please send two slides per FISH test requested, Please refer to the table below with regards to number of slides for a specific referral reason.
- In cases where only part of the tissue is infiltrated, or only part of the tissue is appropriate for screening, please provide an H&E with the relevant area marked. In the absence of brightfield stain, it will be assumed that all of the tissue section will be representative of the tumour tissue and a comment will be made on the report.
- Archival material is accepted.
- Send all slides in a protective container together with the referral card and preferably your own Histopathology report. All required fields on the Oncology Cytogenetics laboratory referral card are coloured in blue (see Request Form).
- Please address all samples to Oncology Cytogenetics (see Contact Details).

Examples of slide numbers required for specific test types;

Referral	Minimum number of slides required (plus at least one spare)
Burkitt lymphoma	4 + 4
Mantle cell lymphoma	1 + 1
Follicular lymphoma	1 + 1
MALT lymphoma	1 + 1
HER2	1 + 1*
ALK	1 + 1
Mesothelioma	1 + 1

*HER2 slides; send extra (up to 6) if IHC or tests other than FISH are required.

HER2 An H&E slide is assessed or ringed area of interest (if provided) to identify areas of disease infiltration. Otherwise any area of the tissue section will be considered representative. The signal pattern is scored in multiple areas (minimum of 4) across the slide. Individual cells need not be scored or reported. For further details about specific requirements for different tumour referrals, please see Paraffin-embedded Tissue for Solid Tumours.

FISH Biological and clinical decision values and reference ranges

Departmental protocols are optimised so that hybridisation of FISH probes to cells on the microscope slides are usually of the highest quality. The hybridisation efficiency to detect rearrangements for specific purposes are evaluated for each test and recorded. If the laboratory feels the ability of a test to detect specific diagnostic features is compromised, the report will be qualified or the test failed.

FISH relies on the presence of disease cells and in diagnostic specimens with heavy disease involvement; this is not usually a problem. Different areas of a slide are analysed and specific areas, as indicated by the referring pathologist, can be preferentially analysed if indicated (see above). Caution is exercised in reporting specimens that are not considered representative of the disease and reports will be qualified appropriately.

Different FISH probes hybridise to different sequences in the DNA, of different lengths and using different strategies to detect different genomic features. Each reagent, therefore, has different performance parameters for the detection of specific abnormalities, also depending on the type of tissue being analysed and disease stage. Some cut-off levels are published for certain disease type (e.g. see CLL and myeloma). The laboratory collects control data on normal cases for each FISH probe, to understand test performance and to evaluate the sensitivity of the tests (protocol Calculating FISH false positive rates from controls [LI-CG-CPP-FISH controls]). Different false positive background levels are established for each probe to inform probe performance for different applications, which will be reported on the individual report, as required.

Email and Telephone Enquiries

Email enquiries are preferred and requests should be made to our departmental email address, which is monitored regularly. Cytogenetics is pleased to accept requests to process samples for specific dates and will make every effort to make results available. We welcome notification of samples arriving, requests to activate samples for analysis, requests for additional tests or result enquiries, which will be processed at the earliest opportunity.

Please make telephone enquiries for urgent results only, if required to determine patient management.
If the Duty Scientist is not available to speak to immediately, please leave a message with Cytogenetics' Secretary or on Voicemail and your call will be returned as soon as possible.

Requests for additional tests (add-ons)

Requests for additional tests on a sample, that are within the scope of the original request (e.g. cytogenetic analysis or FISH), are part of the routine work-up of a case and are accepted by telephone or by e-mail from the referring consultant or other clinician in the care team. A new hard-copy written request for each additional test is not required but the details of the name of requestor and details of the time and date of the request will be recorded on the laboratory database. Some referrals are held "in abeyance" pending further clinical information (see Sample Prioritisation and Reporting Times) and can be activated in the same way. Requests for tests not within the remit of the original investigations (such as on-referral for molecular genetics studies) will require a new, signed request card to accompany the sample. The relevance of the additional test and suitability of the remaining specimen for testing will be reviewed by the handling clinical scientist or consultant clinical scientist and discussed with the requestor, if necessary. New tests will be prioritised in the order the sample was first received. Any additional costs for extra testing will be charged to the original referring hospital.

Reporting

E-mailing Reports

The department routinely e-mails encrypted reports to workplace e-mail addresses of referring consultants and other designated staff in the care team. Multiple recipients are possible to ensure that all appropriate team members receive the cytogenetic result at the earliest opportunity. E-mails are sent directly from the laboratory database and will contain an attachment of the report in pdf format. This will be an unsigned pdf copy of the final report. Reports are encrypted by the Trust's secure e-mail portal. This will require that a username and password are set up online at first use. Thereafter, reports can be accessed by clicking on the attachment and entering your usual password.

By special arrangement, email reports can be sent to an nhs.net address of a member of the patient's care team (if they are not on the normal distribution list) from the Oncology Cytogenetics nhs.net account (see Contact Details). This is not automated and is not currently for regular use.

Reporting to SIHMDS Databases for Integrated Reports

A number of North West regional Haematology services refer specimens to Specialist Integrated Haematology Malignancies Diagnostic Services (SIHMDS) in Manchester (GM HCDP) and Leeds (HMDS Leeds) for the diagnosis of neoplastic diseases of the bone marrow. These SIHMDS services use Oncology Cytogenetics at The Christie as their cytogenetics service provider.

The Oncology Cytogenetics laboratory has access to SIHMDS online databases and we monitor daily for samples referred from participating hospitals. Depending on the reason for referral, cytogenetic analysis is either performed automatically or we may use morphology and flow results to inform the best handling and analysis of our sample. We also respond to the haematopathologist's requests for cytogenetics or FISH studies. The SIHMDS information allows prioritisation of our specimen and ensures the correct cytogenetic or FISH tests are carried out. Occasionally Cytogenetics' samples are not analysed but can be reactivated at a later date.

Upon completion, the cytogenetics report is issued as hard copy and e-mailed to the consultant, as usual. A summary of the results is added to the SIHMDS online combined report, quoting The Christie Cytogenetics laboratory number. Also, a PDF of the full cytogenetics report is uploaded to HMDS Leeds and can be found in the Data Files menu for the sample, under our lab. no. The cytogenetics and FISH reports that are present on the HMDS Leeds database are those from The Christie and tests are not duplicated. This facility allows for cytogenetic test results to aid the diagnostic interpretation and to inform the integrated report.

Hard-copy Reports

Signed hard-copy reports are no longer issued routinely by the Oncology Cytogenetics laboratory. Results are sent by secure email and electronically to SIHMDS databases (see above). Signed hard copy reports will be sent to referring consultants if we do not hold a relevant workplace email address for you and if the referral is non-haematological without a corresponding record on a SIHMDS database.

Policy for Faxing Reports

Faxing of reports was discontinued in March 2020, in accordance with NHSE policy. Our preferred method of reporting is by encrypted email or between nhs.net accounts (see above).

Contacting the Laboratory and Complaints Procedure

The department is keen to encourage service users to provide feedback, as a means of helping us to continually review and improve the services which we provide. We particularly welcome suggestions for new tests and services to help us provide a fully comprehensive genomics service to patients.

The laboratory issues an annual electronic Service User Questionnaire using Survey Monkey® and we are grateful for your rating and comments. All returns are given careful consideration and a report will be issued in response by the Quality and Governance Manager of the Christie Pathology Partnership. However, please feel free to contact us at any time with suggestions (see Contact Details)

We hope that customers of the Oncology Cytogenetics are fully satisfied with the service they receive. If you feel dissatisfied or have concerns with any elements of the service, we would appreciate you contacting the Consultant Clinical Cytogeneticist or if you prefer the Quality and Governance Manager of the Christie Pathology Partnership (CPP).

The Oncology Cytogenetics department will handle formal complaints and concerns according to the **Complaints and Concerns Policy** of the CPP (QI-PathGen-CPP-Complaints). This is a documented process for listening, responding and making improvements when service users, patients and their relatives or carers raise concerns/complaints. This ensures an appropriate and thorough investigation of the issues will be performed, within specified timescales, and that the issue will be resolved promptly with a relevant response to the customer.

Summary of Services Offered by conventional Cytogenetics and FISH for haematological malignancies

Disease group	Cytogenetics	FISH
Chronic Myeloid leukaemia (at diagnosis)	✓ Full analysis for Ph and additional cytogenetic abnormalities (ACA)	✓ (BCR/ABL1)
Chronic myeloid leukaemia (follow-up)	✓ Screen for Ph and ACA including for Ph –ve clones	✓
Acute myeloid leukaemia (at diagnosis) **APML** (at diagnosis)	✓ ✓	To confirm specific abnormality found by cytogenetics or as indicated by morphology, such as CBFB or RUNX1/RUNX1T1. del(5q), del(7q) and del(17p), TP53 on failed specimens. KMT2A in monoblastic AML *__Rapid FISH panel upon request__* Paediatric cases; NUP98, KMT2A and other rare rearrangements for MyeChild01 **Rapid FISH for PML/RARA**. RARA FISH for variants if negative.
MDS	✓ Microarray on failed karyotypes	del(5q) and del(7q) on failed specimens. Other FISH as indicated. Additional FISH for secondary disease.
MPN	✓	Typically only after mutations (JAK2, CALR and MPL) have been investigated. Karyotype if ?transformation
ET	X (on request only)	BCR/ABL1 on request only. Patients where atypical morphology indicates possible CML
PRV	X (on request only)	X
PMF	✓ Karyotype to aid prognostication in DIPSS+ for PMF	FISH panel for prognostic markers on request
CMML	✓	X
Hypereosinophilia	✓	FIP1L1-PDGFRA. PDGFRB, FGFR1, JAK2 FISH as indicated.
ITP	X (on request only) ✓ paediatric	X
Aplastic anaemia	✓	FISH for 5q, 7q, 13q deletions monosomy 7, trisomy 8
BMT patients (sex-mismatched)	Only if % recipient cells is rising/significant by FISH.	✓ (X/Y)
BMT patients (sex-matched)	✓ (if abnormal at diagnosis)	✓ (if abnormal at diagnosis)
All follow-ups (except CML)	✓ (if abnormal at diagnosis)	✓ (if abnormal at diagnosis)
Acute lymphoblastic leukaemia	✓ Microarray analysis	ALL panel: BCR/ABL1, KMT2A, ETV6/RUNX1 (paediatric). TCF3, Hypodiploidy and hyperdiploidy panels if indicated. ABL1, ABL2, PDGFRA, PDGFRB if others negative or if poor response to induction therapy.
CLL/SLL	X	✓ TP53 and ATM only

T Prolymphocytic Leukaemia	✓	✓ TCL1, chr 8
Multiple Myeloma (confirmed) [not MGUS]	X	CD138 cell enrichment. Routinely 1p/1q, TP53 and IGH, followed sequentially by (as indicated) IGH/FGFR3, IGH/CCND1, IGH/MAF, IGH/MAFB
Fanconi Anaemia (screening for leukaemic clones only)	✓ (BM Only)	MECOM (EVI1) FISH (BM) MECOM & 7q FISH (PB)
Other neoplastic diseases with bone marrow involvement e.g. solid tumours	X (on request, if disease present and cytogenetics would be informative)	X (on request, if disease present and FISH would be informative or if FISH is abnormal on other tissue)
Lymphoma (on staging bone marrow aspirate)	X (on request, if lymphocytosis) ✓ If Burkitt lymphoma with marrow involvement	X (on request if lymphocytosis or if FISH abnormal on other tissue)

Lymphoma (on paraffin embedded tissue) various subtypes (FISH only)	
Burkitt Lymphoma	MYC, IGH-MYC, IGK, IGL, BCL2, BCL6
Burkitt Like Lymphoma with 11q Abnormalities	11q copy number FISH
Large B Cell Like Lymphoma with IRF4 Rearrangement	IRF4
High Grade Lymphoma / DLBCL	MYC, IGH-MYC, IGK, IGL, BCL2, BCL6
Mantle Cell Lymphoma	IGH-CCND1, CCND1, CCND2
ALK Positive Large B Cell Lymphoma/ ALK Positive Anaplastic Large Cell Lymphoma	ALK
Follicular Lymphoma	IGH-BCL2 (BCL2/BCL6 as requested)
MALT-Lymphoma	MALT1
ALK Negative Anaplastic Large Cell Lymphoma	IRF4/DUSP22, TP63
Hepatosplenic T Cell Lymphoma	i7q rearrangement FISH

Summary of Services Offered by FISH on paraffin-embedded tissue for solid tumours

Disease group	FISH
Breast carcinoma (with Breast Tumour Receptor laboratory)	IHC and FISH for HER2 amplification status
Oesophagogastric carcinoma (with Breast Tumour Receptor laboratory)	IHC and FISH for HER2 amplification status
Oligodendroglioma and other brain gliomas	1p & 19q codeletion status. Service was withdrawn in 2021. Available from North West Genomics Laboratory Hub (GLH)
Renal cell carcinoma	TFE3 gene fusions at Xp11
Non-Small Cell Lung Cancer	ALK Service was withdrawn in 2021. Available from North West Genomics Laboratory Hub (GLH)
Mesothelioma	Homozygous deletion of CDKN2A
Adenoid Cystic Carcinoma	MYB
Secretory Carcinoma (Salivary Gland)	ETV6, NTRK3
Mucoepidermoid carcinoma	MAML2, NTRK3
Sarcoma - various subtypes	
Round Cell Sarcoma of Soft Tissue Differential	DDIT3, EWSR1, FOXO1
Spindle Cell Soft Tissue Tumour Differential	EWSR1, FUS, MDM2 amp, SS18, COL1A1-PDGFB, ALK
Myxoid Soft Tissue Tumour Differential	DDIT3, FUS
Adipocytic Soft Tissue Tumour Differential	DDIT3, MDM2 amplification
Epithelioid Soft Tissue Tumour Differential	EWSR1, FUS, SS18, TFE3
Ewing sarcoma and other tumours	EWSR1, EWSR1-FLI1
Clear Cell Sarcoma of Soft Tissue	EWSR1
Myoepithelial Tumours of Soft Tissue	EWSR1
Synovial sarcoma	SS18. SS18-SSX1
Liposarcoma/low grade fibromyxoid sarcoma	DDIT3, FUS, MDM2 amplification
Low Grade Fibromyxoid Sarcoma, Sclerosing Epithelioid Fibrosarcoma	FUS
Myxoid/round cell liposarcoma	DDIT3
De-differentiated and well differentiated liposarcoma	MDM2 amplification
Alveolar rhabdomyosarcoma (ARMS)	FOXO1, PAX3/7
Dermatofibrosarcoma protuberans (DFSP)	COL1A1-PDGFB
Alveolar soft part sarcoma	TFE3
Uterine Sarcomas (inc Endometrial stromal sarcoma)	JAZF1, YWAHE, PHF1
Inflammatory Myofibroblastic Tumour	ALK

SERVICE SPECIFICATIONS AND INDICATIONS

CONVENTIONAL CYTOGENETIC TESTING AND FISH FOR HAEMATOLOGICAL MALIGNANCIES

FISH ON PARAFFIN-EMBEDDED TISSUE FOR SOLID TUMOURS

CONVENTIONAL CYTOGENETIC TESTING AND FISH FOR HAEMATOLOGICAL MALIGNANCIES

CHRONIC MYELOID LEUKAEMIA (CML)

Service Provided

- A full karyotype at diagnosis to detect t(9;22) and any additional cytogenetic abnormalities (ACA). Some ACA are now considered high risk baseline prognostic factors. Diagnostic samples will be treated urgently and a result will be available within 7 days.
- FISH at diagnosis to detect BCR-ABL1 gene rearrangement. FISH is performed on all cases at diagnosis, This can be offered rapidly if required, for confirmation of BCR/ABL1 status if this has not already been confirmed. This also establishes a signal pattern for possible future monitoring. FISH will detect the BCR-ABL1 gene rearrangement in cases with normal cytogenetics, with variant translocations involving other chromosomes and in the small number of cases where cytogenetics fails.
- Monitoring response to treatment should be performed by qPCR at the molecular genetics laboratory and assessed according to the International Scale (IS). Post-treatment bone marrows received for cytogenetics will be screened for Ph positivity and BCR/ABL1 by FISH. Conventional cytogenetic analysis will also screen for 'major route' ACA. Ph negative cells are screened for common abnormalities found in Ph negative clones.
- Cytogenetics should be performed post-treatment with atypical translocations, rare or atypical transcripts that cannot be measured by qPCR.
- Conventional cytogenetic analysis on peripheral blood, to monitor remission, is unlikely to be successful.
- Full karyotype at treatment failure/resistance or suspected progression to Accelerated Phase analysed for t(9;22) and ACA

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical CML cases are useful for FISH testing but may not yield sufficient cells for full cytogenetic analysis.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis if required	3
Cytogenetic analysis (karyotype)	7
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at treatment failure/progression	7

References;
European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. A. Hochhaus et al, Leukemia volume 34, pg 966–984 (2020)

ACUTE MYELOID LEUKAEMIA (AML)

Service Provided

- Full karyotype at diagnosis. Classification of AML according to WHO 2022 or ICC 2022 Classifications, with recurrent genetic abnormalities, AML myelodysplasia-related (AML-MR) and therapy related myeloid neoplasms.
- Prognosis according to standard systems for adult and paediatric referrals. To be used in conjunction with gene mutations for ELN 2022 genetic risk stratification.
- Rapid FISH for t(15;17) at diagnosis (usually <24 hours) in APML.
- Extensive rapid FISH panel at diagnosis to characterise CBF-AML, high risk disease and AML-MRC, if required for patient management or if karyotype fails
- Specific FISH at diagnosis as indicated by morphology, where conventional cytogenetics fails and to aid the detection of secondary AML. Special FISH panel in paediatric AML for Myechild01 trial
- Upload of diagnostic results to central database for MRC AML trials and MyeChild01
- Post-treatment bone marrows screened for previous abnormality by routine cytogenetics or FISH, as appropriate
- Full karyotype at suspected relapse for recurrence of previous abnormality, clonal evolution or new disease.

Favourable prognosis	t(8;21)(q22;q22.1)/RUNX1::RUNX1T1 inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11
Intermediate prognosis	t(9;11)(p21.3;q23.3)/MLLT3::KMT2A Cytogenetic and/or molecular abnormalities not classified as favourable or adverse
Adverse prognosis	t(6;9)(p23.3;q34.1)/DEK::NUP214 t(v;11q23.3)/KMT2A-rearranged t(9;22)(q34.1;q11.2)/BCR::ABL1 t(8;16)(p11.2;p13.3)/KAT6A::CREBBP inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM (EVI1) t(3q26.2;v)/MECOM (EVI1)-rearranged -5 or del(5q); -7; -17/abn(17p) Complex karyotype,** monosomal (≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities or hyperdiploidy)

2022 ELN risk classification by genetics at initial diagnosis. Adapted from Döhner H et al. Blood. 2022 Sep 22;140(12):1345-1377. Please see full article for full Table including molecular abnormalities

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect.

Following rapid FISH for diagnosis of APML, a full karyotype will be performed to look for t(15;17) or other chromosomal abnormalities. Further FISH would be required in negative cases, to look for variant translocations where APML is still suspected. Molecular studies may be required to test for rare submicroscopic insertional PML-RARA gene fusions.

Sample requirements

Bone marrow aspirate or peripheral blood specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis e.g. PML-RARA	3 (typically within 1 working day)
Urgent karyotype and FISH at diagnosis if required for treatment options	5
Cytogenetic analysis / other diagnosis (karyotype)	7
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at ?relapse or transformation	14

References;

- Khoury JD et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719.
- Arber DA et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022 Sep 15;140(11):1200-1228
- Döhner H et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377

MYELODYSPLASTIC SYNDROMES (MDS)

Service Provided

- Samples are accepted on all cases of suspected MDS where bone marrow examination is indicated.
- Full cytogenetic analysis will be performed to test for clonal abnormalities of diagnostic and prognostic significance.
- SNP microarray on samples where karyotype fails following cytogenetic culture. FISH for 5q and 7q deletions and an extended FISH panel in secondary MDS for TP53, KMT2A and MECOM gene rearrangements is available if further testing is still required and for balanced translocations.
- Storage only on samples with macrocytic anaemia and other cytopaenias, pending confirmation of diagnosis of MDS. If reactive causes have been excluded and persistent cytopaenia are of undetermined origin, please contact the laboratory to request analysis.
- Periodic re-investigation of sequential bone marrows, particularly if there is change in clinical or laboratory findings.

Cytogenetic risk groups in IPSS for MDS (Greenberg PL et al. (2012) Blood Sep 20;120(12):2454-65).

Very good prognosis	-Y, del(11q)
Good prognosis	Normal karyotype, del(5q), del(12p), del (20q). double including del(5q)
Intermediate prognosis	del(7q), + 8, +19, i(17q), any other single or double independent clones
Poor prognosis	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex karyotype (= 3 abnormalities)
Very poor prognosis	Complex karyotype (>3 abnormalities)

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines.
Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical MDS cases are unlikely to be useful.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype) of new MDS	21
FISH on the above	21
Cytogenetic analysis of MDS ?transforming to AML	7
Cytogenetic analysis of ?secondary MDS	14

APLASTIC ANAEMIA (AA)

Service Provided

- Full cytogenetic analysis for clonal abnormalities of diagnostic and prognostic significance. Cytogenetics may aid the distinction of AA from hypoplastic MDS or indicate clonal evolution to MDS or AML.
- Detection of high-risk chromosomal abnormalities, particularly monosomy 7 and complex karyotypes.
- FISH for 5q, 7q and 13q deletions, monosomy 7 and trisomy 8 on samples that fail to grow in cytogenetic culture.

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Low cellularity of the aspirates in AA often leads to small numbers of mitotic cells to analyse and therefore unsuccessful cytogenetic testing. Peripheral blood specimens from typical AA cases are unlikely to be useful.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype) of AA	21
FISH on the above	21
Cytogenetic analysis of AA ?transforming to MDS	14

References. Killick et al. Br J Haematol. 2016 Jan;172(2):187-207.

MYELOPROLIFERATIVE NEOPLASMS

Service Provided

- Current recommendations require investigation of Ph-negative classical MPN by molecular testing for JAK2 V617F (and CALR and MPL mutations in ET and JAK2 exon 12 mutation in PRV), before referral for cytogenetic analysis. Molecular testing is available from the GLH at MFT (see Cross et al, 2021).
- A full cytogenetic analysis and/or FISH can be performed when reactive causes of myeloproliferation have been excluded and may help to confirm clonality, aid differential diagnosis and indicate prognosis in certain contexts.
- Testing for BCR::ABL1 gene fusion is offered in ET with atypical morphology for exclusion of CML.
- Karyotype is offered at suspected disease transformation. Whilst there is no specific abnormality that will confirm transformation, karyotype complexity and abnormalities such as del(5q), monosomy 7 or del(17p) are highly suggestive of progression.
- Cytogenetic analysis is included in diagnostic algorithms for Primary Myelofibrosis (see below). A cytogenetic risk score for inclusion in the DIPSS Plus (or other prognostic algorithms) can be reported. FISH for prognostic markers is available on request, in the event of karyotype failure.

Risk	Cytogenetic Abnormality
Favourable	Normal karyotype or sole abnormalities of 20q-, 13q- or +9. Chromosome 1 translocation/duplication or sex chromosome abn including –Y
Unfavourable	All other abnormalities.
Very high risk	Single/multiple abnormalities of −7, i(17q), inv(3)/ 3q21, 12p, 11q−/11q23, or other autosomal trisomies not including + 8/ + 9 (e.g., +21, +19)

Adapted from Tefferi A et al. Leukemia. 2018 May;32(5):1189-1199 used in DIPSS+ for myelofibrosis

- Full karyotype to aid diagnosis of CMML is available with BCR-ABL1 FISH testing on request. Cytogenetic risk for stratification will be reported according to CMML-specific prognostic scoring system (CPSS) which includes trisomy 8, abnormalities of chromosome 7 and complex karyotype (>3 chromosomal abnormalities) as high risk (Such E et al. Blood. 2013 Apr 11;121(15):3005-15).
- Full conventional cytogenetic analysis and FISH is available for investigation in patients with hypereosinophilia (after reactive cases and other diseases associated with eosinophilia have been excluded). FISH for FIP1L1-PDGFRA fusion can be performed as a front line test on peripheral blood. Full karyotype and/or FISH for PDGFRA, PDGFRB, FGFR1 and JAK2 rearrangements are available to aid diagnosis of myeloid/lymphoid neoplasms with eosinophilia and specific genetic abnormalities.
- A full cytogenetic analysis is not indicated for referrals with Systemic Mastocytosis as specific diagnostic chromosome abnormalities are not recognised. Screening for KIT D816V mutation is available from the GLH at MFT.

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical MPN cases may be used if immature cells are present. A fresh bone marrow trephine specimen is an option, if the marrow is fibrotic or otherwise difficult to aspirate.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype)	21
FISH	21
Cytogenetic analysis of MPN ?transforming to AML	7

References.

Cross NCP et al; 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.

Barbui T et al. ELN recommendations. Leukemia. 2018 May;32(5):1057-1069.

Tefferi A et al. Am J Hematol. 2019 Jan;94(1):133-143. Vainchenker W and Kralovics R. Blood. 2017 Feb 9;129(6):667-679.

ACUTE LYMPHOBLASTIC LEUKAEMIA & LYMPHOMA (PRECURSOR LYMHOID NEOPLASMS)

Services Offered

- SNP microarray and FISH at diagnosis. Classification of B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities according to WHO 2022. Full karyotype is also attempted. Cytogenetics should be carried out at diagnosis in all cases.
- Rapid FISH for BCR-ABL1 and KMT2A gene rearrangements (and ETV6-RUNX1 and iAMP21 in children)
- Subsequent, sequential FISH at diagnosis for TCF3-PBX1, TCF3-HLF, specific KMT2A translocations, as required.
- Detection of all relevant cytogenetic abnormalities to provide risk stratification for different demographic subsets (adults, children and infants) and to support AllTogether01, UKALL14 and UKALL2011 trials.
- Patients without recognisable cytogenetic abnormalities ("B-other ALL") or poor responders to induction treatment will be screened for 'ABL-class' gene fusions; ABL1, ABL2, PDGFRA and PDGFRB by FISH.
- Post-treatment bone marrows screened for previous abnormality by FISH. Routine cytogenetics will not be used to monitor response to therapy; conventional karyotyping has very limited sensitivity to detect residual disease.
- Full karyotype and relevant FISH at suspected relapse

	Childhood ALL*	Adult ALL**
Good prognosis	t(12;21) [ETV6-RUNX1], high hyperdiploidy	high hyperdiploidy, del(9p)
Intermediate prognosis	Other abnormalities including; t(1;19)(q23;p13) dup(1q) del(6q) -7 Abnormal 9p dic(9;20)(p13;q11) dic(9;12)(p11–21;p11–13) Abnormal 11q	Other abnormalities including; t(1;19)(q23;p13) del(6q) -7 +8 11q23 [KMT2A] translocations other than t(4;11) Abnormal 11q del(12p) Loss of 13q Abnormal 17p
Poor prognosis	t(9;22) 11q23 [KMT2A] translocations iAMP21 [RUNX1 amplification] Near haploidy (<30 chromosomes) Low hypodiploidy (30–39 chromosomes) t(17;19)(q23;p13) Abnormal 17p Loss of 13q	t(9;22) t(4;11) Low hypodiploidy (30–39 chromosomes)/ near triploidy Complex karyotype (≥5 abnormalities)

Adapted from; *Moorman et al 2022[1] ** Moorman et al 2022[2]

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect. ALL disease cells are notorious for their poor survival in vitro and rapid transport to the laboratory is critical for successful chromosome analysis and FISH. ALL

disease cells also often have poor chromosome morphology. The sensitivity of monitoring follow-up cases in remission is therefore likely to be severely limited.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens with disease cell involvement are suitable specimens for diagnosis.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis	3 working days
Cytogenetic analysis (karyotype)	7
Subsequent FISH prognostic markers	14
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at ?relapse or transformation	14

References

1. Moorman AV et al. Time to Cure for Childhood and Young Adult Acute Lymphoblastic Leukemia Is Independent of Early Risk Factors: Long-Term Follow-Up of the UKALL2003 Trial. J Clin Oncol. 2022 Dec 20;40(36):4228-4239.
2. 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.
3. Alaggio R et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022 Jul;36(7):1720-1748.

MATURE B & T-CELL LYMPHOMAS

Services Offered

- FISH to detect gene rearrangements to aid diagnosis of lymphoma subtypes

Lymphoma type	Typical genetic abnormality
Burkitt Lymphoma	MYC, IGH-MYC, IGK, IGL, BCL2, BCL6
Burkitt Like Lymphoma with 11q Abnormalities	11q copy number FISH
Large B Cell Like Lymphoma with IRF4 Rearrangement	IRF4
High Grade Lymphoma / DLBCL	MYC, IGH-MYC, IGK, IGL, BCL2, BCL6
Mantle Cell Lymphoma	IGH-CCND1, CCND1, CCND2
ALK Positive Large B Cell Lymphoma/ ALK Positive Anaplastic Large Cell Lymphoma	ALK
Follicular Lymphoma	IGH-BCL2 (BCL2/BCL6 as requested)
MALT-Lymphoma	MALT1
ALK Negative Anaplastic Large Cell Lymphoma	IRF4/DUSP22, TP63
Hepatosplenic T Cell Lymphoma	i7q rearrangement FISH

- Rapid FISH for identification of MYC gene rearrangements in Burkitt/High grade lymphoma
- 'Burkitt panel' of FISH tests to aid distinction between BL and DLBCL; MYC, IGH/MYC dual fusion, IGH/BCL2, BCL6. IGK and IGL detect immunoglobulin light chain gene variants. Additional abnormalities in the karyotype can also aid diagnosis if fresh, involved tissue is available (see conventional cytogenetics below)
- FISH on paraffin-embedded tissue sections
- FISH and/or conventional cytogenetics on fresh bone marrow, lymph node biopsies or other primary tissue.
- Bone marrow staging samples are accepted but will only be analysed if they are shown to be infiltrated by disease, confirmed by other methods. Until confirmation of disease involvement, samples will be stored pending further information. Generally, disease subtypes without a diagnostic cytogenetic hallmark or if patient management is not affected, will not be analysed
- Once a gene fusion is detected by FISH at diagnosis, this test can be used to monitor future samples for residual disease, possible relapse and infiltration of secondary tissue.

Technical

FISH is the gold standard routine test for detection of translocations in lymphoma, and is standard procedure used in this laboratory. It can be used on fixed fresh cells from bone marrow and blood or on FFPE tissue sections.

FISH is used to detect MYC rearrangements in high-grade lymphoma; the department uses a wide-gap FISH assay to maximise the sensitivity of MYC breakpoint detection. However, due to the wide variation in breakpoint location within the MYC region, particularly when a gene other than IGH is the partner, rare breakpoints rearrangements may not be detected.

Conventional cytogenetic analysis is performed on cells from fresh bone marrow, using unstimulated overnight, and 72 hour cultures and B- or T-cell mitogen stimulated cultures as appropriate. The karyotype from up to 40 cells from different cultures will be analysed, according to standard procedures and best practice guidelines. Cells may contain

cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. FFPE tissue sections can be sent for FISH and is described above (see Sample requirements for Paraffin-embedded Tissues)

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid MYC FISH at diagnosis of Burkitt lymphoma	3 working days
Cytogenetic analysis (karyotype) and subsequent FISH in Burkitt/high grade lymphoma	7
FISH or cytogenetics for other lymphoma diagnosis	14
FISH or cytogenetics for lymphoma staging marrows	21

CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)/ SMALL LYMPHOCYTIC LYMPHOMA

Services Offered

- FISH for TP53 and ATM gene deletion in blood and bone marrow samples with lymphocytosis, in patients with CLL about to undergo treatment
- Blanket FISH testing for IGH-CCND1 for the exclusion of mantle cell lymphoma is not performed routinely. Please request IGH/CCND1 FISH to detect t(11;14) in all cases of CLL with atypical morphology or with a CLL immunophenotype score of 3 or less.
- Full karyotyping is not routinely performed, but is available, by special advance request to aid the differential diagnosis of other lymphoproliferative disorders

Referrals

There is no evidence that treatment of early CLL improves overall outcome and so FISH testing is indicated only prior to first treatment. Chromosomal abnormalities may develop during disease course and FISH analysis should also be considered prior to subsequent treatments.
FISH tests are not suitable for monitoring remission. Referred samples must be from involved tissue with significant lymphocytosis. Peripheral blood is suitable test material, in most cases. CLL patients suspected of secondary myeloid disease need to be highlighted as they will be handled differently.

Technical

FISH for TP53 and ATM deletions is a standard procedure and the test of choice.
FISH for deletion has a high false positive background; levels of less than 10% for TP53 deletion and less than 5% for ATM deletion are not considered significant and will not be reported. Levels within 5% above these cut-offs will be considered borderline and reported suggesting a need to confirm the result in a later sample (e.g. ATM 5~10% and TP53 10~15%).

Sample requirements

Bone marrow aspirate or peripheral blood specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
FISH for 17p/TP53 and 11q/ATM deletions. Cell culture and storage of bone marrow cells in case conventional cytogenetic analysis indicated at a later date	7
FISH for IGH-CCND1 by request/indication	7 days, if indicated
Full FISH panel (plus 13q and trisomy 12)	Not routine. By special request only
Conventional cytogenetic analysis	Not routine. Please enquire

MYELOMA

Services Offered

- Enrichment of CD138 expressing cells by immunomagnetic separation, as indicated by referral reason
- FISH on CD138-enriched bone marrow preparation to identify the adverse risk abnormalities; t(4;14) (IGH/FGFR3), t(14;16) (IGH/MAF), t(14;20) (IGH/MAFB), 1q gain, del(1p) and TP53 deletion. The FISH strategy will also identify the common standard risk abnormality t(11;14) (IGH/CCND1), which can aid counselling and as a possible biomarker for targeted treatment.
- Reports will advise on prognosis for incorporation into International Staging System (ISS) to identify patients with high-risk myeloma (according to NICE Myeloma: diagnosis and management guidelines 2016)
- t(6;14) (IGH/CCND3), MYC rearrangements, and copy number FISH for Hyperdiploidy are available in the National Genomic Test Directory for cancer, however, are not provided in our sequential strategy for high risk cytogenetics. Please enquire if these tests are required for patient management.

Cases are tested for IGH rearrangements using IGH breakapart probe plus 1p/1q and TP53 (3 tests). If a. If IGH positive, tests for specific partner genes are performed sequentially in order of prevalence. If IGH negative, there are no further tests.

Neutral prognosis	t(11;14)
Poor prognosis	t(4;14); IGH/FGFR3 t(14;16); IGH/MAF t(14;20); IGH/MAFB TP53 deletion Gain of 1q21.3 (CKS1B)/deletion 1p32.3 (CDKN2C)

Conventional cytogenetic analysis is not routinely performed in myeloma. Conventional cytogenetic analysis is hampered by the low proliferation rate of myeloma cells in culture and a number of the abnormalities being cryptic. Cells are cultured to investigate other diseases or secondary myeloid disease if indicated; please contact the laboratory to request this.

- Repeat FISH testing at relapse, for risk re-stratification. A full panel of FISH tests will be performed at relapse, to look for new emerging clones that were not detected at diagnosis. If a patient already has an identified high-risk feature at diagnosis, then there may be no need to perform repeat investigations at relapse.

Referrals

The FISH service tests for prognostic markers at diagnosis or relapse. Analysis is applicable for symptomatic cases of confirmed myeloma only. Cases with an uncertain diagnosis will be stored until a diagnosis of myeloma is confirmed, so that unnecessary tests are not performed on MGUS, in which the abnormalities do not have the same significance. Please contact the laboratory to activate a case following diagnosis.

FISH testing is not suitable to monitor disease course and remission samples cannot be tested. Myeloma patients suspected of secondary disease need to be highlighted as they will be handled differently.

Technical

Where myeloma is indicated on the referral card and sufficient cells are available, FISH is performed on CD138 separated cells and so disease plasma cells should be enriched in the sample. Rare CD138 negative cases will not be enhanced. The cut-off levels above which an abnormal clone can be confirmed is ≥ 10% for dual fusion abnormalities and ≥ 30% for TP53 deletion and single fusion patterns (modified from EMN Guidelines; Ross et al, Haematologica 2012). Cut-offs for 1p deletion and 1q gain are 20%. Please note; the laboratory protocol involves storage of cells for one week after fixation, which has been shown to enhance the quality of FISH post CD138 cell

separation and reduce false-positive background. Normally results will not be available within one week and will be reported in 2 – 3 weeks.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Peripheral blood samples are nor appropriate for myeloma referrals unless the patient has peripheral involvement (Plasma cell leukaemia). Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. To allow time for cell separation and sample processing all samples should be received before 3pm on Friday.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
5 FISH panel; tested sequentially for IGH, TP53, del(1p)/gain 1q21. Followed by IGH-FGRF3, IGH-CCND1, IGH-MAF, IGH-MAFB, as indicated. Cell culture and storage of bone marrow cells in case conventional cytogenetic analysis indicated	21
Conventional cytogenetic analysis	Not routine. Please enquire

References

NICE (2016). NICE guideline [NG35]. Myeloma: diagnosis and management. https://www.nice.org.uk/guidance/ng35 Updated October 2018.

Sonneveld P, et al. (2016). Treatment of multiple myeloma with high risk cytogenetics: a consensus of the International Myeloma Working Group. Blood 16;127(24):2955-62.

Caers J, et al. (2018) European Myeloma Network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when. Haematologica 103(11): 1772–1784

Rajkumar, S.V. (2018). Multiple myeloma: 2018 update on diagnosis, risk-stratification, and management. American Journal of Haematology 93(8):1091-1110.

FISH ON PARAFFIN-EMBEDDED TISSUE FOR SOLID TUMOURS

BREAST and GASTRIC CARCINOMA (IHC and HER2 FISH)

Services Offered

- The Breast Tumour Receptor laboratory performs immunohistochemistry (IHC) on paraffin embedded tissues for breast receptors, including HER2, ER, PgR, Ki67 and Androgen Receptor
- HER2 IHC cases showing >10% strong, complete membrane staining of invasive cancer cells are regarded as HER2 positive.
- Less than 10% of cells with strong complete membrane staining or greater than 10% of invasive cells are with moderate membrane staining are forwarded for assessment by FISH.
- HER2 FISH, using approved dual colour assay, to determine HER2 status for the use of adjuvant trastuzumab (Herceptin) therapy, in support of NICE guidance in early breast cancer
- Current HER2 testing guidelines are followed which recommend a two-tier service using IHC to detect HER2 protein expression with analysis of equivocal HER2 (2+) cases by FISH to detect gene amplification. Referrals for FISH only are welcome
- HER2 testing in advanced gastric or gastroesophageal cancer by IHC and FISH. **Please refer all cases of gastric tumours directly from the pathologist to avoid delay in testing.** Further information on arrangements for HER2 testing can be obtained from Dr Mansoor, Medical Oncologist at The Christie, at was.mansoor@christie.nhs.uk
- **Enquiries regarding testing for all cell markers by IHC or FISH are welcome.** The Breast Tumour Receptor laboratory offers non-routine testing by special request for trials and other research

Technical

HER2 signals are counted in 20 to 60 non-overlapping invasive cancer cell nuclei by at least two analysts, over three or more distinct tumour fields. Additional cells are scored in borderline cases. A ratio of 1.80-1.99 or a ratio <2.0 and average HER2 gene copy number between 4.0 and 6.0 will be reported as 'Borderline but Not Amplified' and include a clear statement that the tumour is HER2 Negative. A ratio of >2.0 and/or mean HER2 copy number >6.0 will be reported as Positive. Monosomy or partial monosomy of chromosome 17 leading to a dual probe ratio >2.0 will be reported as 'Positive with Monosomy' (Rakha EA et al. J Clin Pathol 2014;0:1-7). Aneusomy i.e. deletions / gains (polysomy) are common in breast cancer and measurement of chromosome number is critically important (ref Walker et al, J Clin Pathol 2008) and is performed in this FISH assay.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Immunohistochemistry	7 days
FISH	14

Turnaround times are assessed as time of receipt in the laboratory to release of report for HER2 IHC & HER2 FISH. Turnaround times are dependent on the time taken for the sample to reach the laboratory from the requestor.

SARCOMA

Services Offered

- FISH to detect gene rearrangements to aid diagnosis of sarcoma subtypes

Sarcoma type	Typical genetic abnormality
Round Cell Sarcoma of Soft Tissue Differential	DDIT3, EWSR1, FOXO1
Spindle Cell Soft Tissue Tumour Differential	EWSR1, FUS, MDM2 amp, SS18, COL1A1-PDGFB, ALK
Myxoid Soft Tissue Tumour Differential	DDIT3, FUS
Adipocytic Soft Tissue Tumour Differential	DDIT3, MDM2 amplification
Epithelioid Soft Tissue Tumour Differential	EWSR1, FUS, SS18, TFE3
Ewing sarcoma and other tumours	EWSR1, EWSR1-FLI1
Desmoplastic small round cell tumour	EWSR1 t(11;22)
Clear Cell Sarcoma of Soft Tissue	EWSR1 t(12;22)
Chondrosarcoma	EWSR1 t(9;22)
Myoepithelial Tumours of Soft Tissue	EWSR1
Synovial sarcoma	SS18. SS18-SSX1 for t(X;18)
Liposarcoma/low grade fibromyxoid sarcoma	DDIT3, FUS, MDM2 amplification
Low Grade Fibromyxoid Sarcoma, Sclerosing Epithelioid Fibrosarcoma	FUS
Myxoid/round cell liposarcoma	DDIT3, FUS t(12;16)
Atypical lipomatous tumours, well-differentiated liposarcoma or dedifferentiated liposarcoma	MDM2 with control for amplification
Alveolar rhabdomyosarcoma (ARMS)	FOXO1, with PAX3 and PAX7 FISH as indicated to detect t(2;13) and t(1;13)
Dermatofibrosarcoma protuberans (DFSP)	COL1A1-PDGFB
Alveolar soft part sarcoma	TFE3
Uterine Sarcomas (inc Endometrial stromal sarcoma)	JAZF1, YWAHE, PHF1 (enquire)
Inflammatory Myofibroblastic Tumour	ALK

Technical

FISH is performed using validated commercially available reagents and are verified in house, for use with our standard protocols. An H&E slide is assessed or ringed area of interest (if provided) to identify areas of disease infiltration. Otherwise any area of the tissue section will be considered representative. The signal pattern is scored in multiple areas (minimum of 4) across the slide. Individual cells will not be scored or reported.

FISH is the gold standard routine test for detection of translocations and other genomic features in sarcoma, and is standard procedure used in this laboratory. However, some studies show that a small proportion of patients within different sarcoma subtypes harbour genetic abnormalities which may not be detectable using a FISH strategy and will not detect uncommon cases where an alternative genetic mechanism is involved. Where the limitations of an assay are known, this will be stated on the report with an estimation of the sensitivity of the test where appropriate.

Scoring for MDM2 Amplification

Representative fields are examined for cells with additional MDM2 signals. It is not always possible to count individual signals. Significant amplification is usually obvious and signal count in individual cells is not usually scored. However, if the amplification status is equivocal, individual cells will be scored in attempt to obtain a signal ratio, if the quality of hybridisation is suitable. Amplification can be reported when MDM2/CEP12 signal ratio is >2.0 (Weaver et al, Mod Path 2008). Polysomy does not indicate amplification but massive co-amplification of signals would be suggestive. Equivocal and uninterpretable results will have a qualified report.

Scoring for COL1A1/PDGFB rearrangement in DFSP

Please note that we do not currently have false positive cut-off data for the COL1A1/PDGFB probe. Due to the high false positive background level associated with the single fusion signal pattern associated with the r(17;22) ring chromosome, it is sometimes necessary to apply a second test using a PDGFB breakapart probe in cases with equivocal results. In practice, positive results are usually clear due to high levels of disease infiltration and frequent amplification of the ring chromosome.

Scoring for Renal Cell Carcinoma and Alveolar Soft Part Sarcoma

Scoring with TFE3 breakapart probe. TFE3 is at Xp11 and patterns will differ according to chromosomal sex of the patient. ASPS gives a single fusion pattern only as the result of an unbalanced der(17)t(X;17)(p11;q25).

Sample requirements

The optimal thickness of sections for FISH testing is 3~4 µm. We prefer an H&E slide, with the relevant area marked, to be sent along with the slides for testing in order to focus our analysis appropriately. In the absence of brightfield stain, it will be assumed that all of the tissue section will be representative of the tumour tissue.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
FISH (single tests or in combination for differential diagnosis (see Services Offered above)	7

MALIGNANT MESOTHELIOMA

Services Offered

- CDKN2A FISH to detect homozygous deletion of 9p21 to aid differential diagnosis of malignant mesothelioma from benign mesothelial proliferations

Please note that the absence of homozygous CDKN2A deletion does not exclude a diagnosis of mesothelioma. Various studies have shown the prevalence of homozygous deletion in pleural mesotheliomas to range from 60%-88% (Illei et al 2003, Takeda et al 2010, Chiosea at al 2008, Dacic et al 2008), with the sarcomatoid histological type showing the highest frequency.

Technical

The laboratory has validated this FISH assay for detection of homozygous deletion of CDKN2A in paraffin embedded tissue sections from suspected cases of malignant mesothelioma. Both cellular pleural effusions and tissue biopsies appear acceptable. The proportion of cells considered positive by the laboratory for reporting a homozygous deletion is ≥20%. Hemizygous deletion (loss of single copy of CDKN2A) or apparent monosomy for chromosome 9 (loss of CDKN2A and one control signal) are not sufficiently sensitive to distinguish malignant tumours and will not be reported.

Sample requirements

The optimal thickness of sections for FISH testing is 3~4 µm. For this test it is important that we are provided with information regarding the tumour type, an H&E slide marked with the area of interest for analysis and if possible with an informative IHC slide (e.g. calretinin), to help guide our analysis to the relevant areas.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
FISH	7