

**SIHMDS-AG: Policy for Undertaking Diagnostic Tests in SIHMDS-AG**

**Principle of this Policy**

For the provision of a consistently high-quality Molecular Genetics and Cytogenetics service, it is essential that appropriate samples are sent for testing, and that they arrive in good condition, safely and promptly with the correct documentation.

Clinical review of all examinations undertaken in SIHMDS-AG is done every six months when Clinical Lead of SIHMDS, Clinical Lead of Neuropathology and Lead Clinical Scientist for SIHMDS-AG meet.

**Implementation**

The implementation of this policy is the responsibility of the SIHMDS-AG Section Leads. All staff working within the department are expected to always treat clients with respect and courtesy.

**Who the policy effects**

This policy affects all Scientific, Support and Clinical staff in SIHMDS-AG.

**Procedure of this Policy**

**Service times**

**Weekdays**

Monday to Friday      9am to 5.30pm

**Weekends and Out-of-Hours**

There is no routine service at weekends or out-of-hours; however, samples requiring special attention may be arranged in advance by contacting the department.

**Public Holidays**

The department is staffed on Bank Holiday Mondays, Good Friday and Easter Monday for processing cytogenetic samples set up previously. Samples for molecular genetic analysis will not be processed out of hours unless prior arrangements have been made. Special arrangements are in place at Christmas and New Year, details of which can be obtained by contacting the department.

**Clinical advice**

Clinical advice on ordering of examinations and on further interpretation of examination results is available during core working hours. Please telephone the SIHMDS laboratory office (ext 5771)

**Review of complaints and incidents**

All complaints regarding the implementation of this policy should be directed to the Head of Section. Complaints will then be channelled through the Trust's complaints procedure. An incident should be reported on the Datix system for all adverse events that may impact on patients and/or staff.

**Patient consent**

Informed consent from the patient for the test(s) will be assumed on receipt of the test request. Patients must be made aware that any extracted DNA/RNA will be stored in the

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department. The stored material will not be used for any purpose requiring patient consent as detailed by the Human Tissue Act (HTA), however it may be used for Quality Control or test validation.

**Data protection**

Everyone working for the NHS who records, handles, stores or otherwise comes across patient information has a personal common law duty of confidence to patients and to his or her employer. This duty of confidence continues even after death of the patient or after an employee or contractor has left the Trust.

All staff in SIHMDS-AG work in accordance with the Trust confidentiality policy and undergo regular mandatory training on information governance. The policy is available on request.

**Samples sent to SIHMDS-AG**

Samples should be sent promptly, as delays may result in test failure. This may be due to

- A lack of dividing cells in the sample; this is especially the case for ALL samples.
- The integrity of RNA in the sample starts to deteriorate as soon as the sample is taken, samples for RNA analysis should reach the department as soon as possible. Samples older than 72 hours will be compromised, may lead to analysis failure, and will be rejected.

Samples must be labelled with at least three unique identifiers.

**Samples sent internally from Great Ormond Street Hospital (GOSH)**

Samples are, at present, sent directly to the SIHMDS-AG laboratory or via the Blood Sciences laboratory, located in the Camelia Botnar Laboratories, Great Ormond Street Hospital

**FFPE tissue samples or frozen tumour samples from Histopathology (GOSH)**

Solid tumour samples, either frozen tissue, touch imprints, FFPE sections mounted on slides or FFPE rolled sections, are sent from the Department of Histopathology, Great Ormond Street Hospital, located in the Camelia Botnar Laboratories.

**Bone marrow and blood samples from UCLH**

SIHMDS-AG at GOSH now accepts Blood, bone marrow or DNA samples from ULCH patients on AllTogether trial for Cytogenetic analysis and molecular genetics analyses.

**External Hospital Samples**

Samples from external hospitals are, at present, sent by courier or Royal mail post to the SIHMDS-AG laboratories and are received in the booking-in area Y5023.

Samples should be sent to:

SIHMDS-Acquired Genomics Laboratory  
NHS North Thames Genomic Laboratory Hub  
Great Ormond Street Hospital for Children NHS Foundation Trust  
Levels 5, Barclay House,  
37 Queen Square,  
London WC1N 3BH  
Tel: 020 7405 9200 x5771/ x5755  
Email: [gos-tr.pmu@nhs.net](mailto:gos-tr.pmu@nhs.net)

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**Sample types and transport media**

**Samples for Cytogenetic analysis**

Bone marrow specimens should be collected into a Lithium Heparin tube.

Peripheral blood should be sent in a Lithium Heparin tube.

**Haemato-oncology samples:**

- Cytogenetics: Karyotype testing (chromosome analysis)
  - 1-5 mls whole blood or bone marrow in preservative free heparin for cell culture
  
- Cytogenetics: FISH testing:
  - 1-5 mls whole blood or bone marrow in preservative free heparin for cell culture
  - Smear slides – peripheral blood or bone marrow,
  - FFPE/touch imprint slides,
  - CSF cytopsin slides – if CNS involved
  
- Molecular genetics: RNA and DNA NGS panel testing, targeted molecular testing:
  - 1-5 mls whole blood or bone marrow in EDTA or ACD
  - Frozen mononuclear cells – for nucleic acid extraction only
  - CSF – if CNS involved
  - Solid tissue (FFPE /frozen) – e.g. testicular relapse
  - DNA, RNA, cDNA

**Solid Tumour Samples**

The laboratory accepts fresh tissue biopsies and Fresh fixed paraffin embedded (FFPE) tissue samples. Infiltrated bone marrow samples may be used for testing in exceptional circumstances where a solid tumour sample is not available and only upon request of the clinician. See below for sample-specific and testing requirements:

**Fresh tissue**

Fresh tissue biopsies should be taken into sterile saline; **under no account should formalin** be used. Snap frozen tissue samples are also accepted. Acceptable samples include:

- a) Biopsy material
- b) Tumour excision material

Every care should be taken to ensure that specimens sent to the SIHMDS-AG laboratory contain viable tumour material.

For FISH testing, touch imprint slides should be made from fresh tissue in saline.

For testing of extracted DNA fresh tissue in saline or frozen tissue should be sent.

Samples for RNA extraction should be sent frozen on dry ice to retain the integrity of the RNA. These frozen samples are immediately transferred to the -80°C freezer in the extraction bay, room Y5017 until further processing

**FFPE material**

Fresh fixed paraffin embedded (FFPE) tissue samples are acceptable for FISH and Molecular Genetics studies.

For FISH testing, sections should be cut at 3µm and mounted on a slide.

For molecular genetics testing (i.e. testing of DNA and RNA), rolled FFPE sections should be cut at 5-10µm and a minimum of 5 rolled sections should be placed in a DNase/RNase free 1.5ml microcentrifuge tube. If RNA and DNA are to be extracted at least two aliquots of rolled sections should be sent.

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- FISH testing:
  - FFPE slides/touch imprint slides
- Molecular genetics testing (DNA/RNA):
  - FFPE rolled sections, fresh frozen tissue in saline, infiltrated bone marrow (EDTA)

### **External samples**

Blood and Bone marrow samples should be collected into an EDTA tube for Molecular Genetic studies and a lithium heparin tube for Cytogenetics analysis. They should be sent to SIHMDS-AG as soon as possible, a referral form with a contact name, nhs.net email address and telephone number should be included.

DNA/RNA samples may be sent for Molecular genetic testing, indication of which laboratory extracted the nucleic acids should be included.

### **Other sample types**

Other sample types such as Pleural Fluid, ascetic fluid, cerebrospinal fluid, cytospin cells, bone marrow or blood smears are accepted by prior agreement.

Where samples are received from patients who did not have their diagnostic cytogenetic testing performed at GOSH, every effort should be made to acquire a copy of the diagnostic cytogenetic report.

All samples should be booked in for the relevant cytogenetic and molecular tests. Cultures, nucleic acid extractions and tests are requested by the duty scientist; refer to section 7 – Testing strategy below and to RGS LAB9601: *Duty Scientist role*.

### **Unlabelled Specimens**

Unlabelled specimens are not accepted for non-invasive samples, however; in exceptional circumstances unlabelled invasive specimens that cannot be re-sampled may be accepted. Unlabelled invasive specimens may be verified in the laboratory by the requesting clinician by following RGS SAB9621: *Processing unlabelled invasive samples of bone marrow, solid tumour and Cerebro-spinal fluid*. The unlabelled specimen form RGF SAB0010: *Disclaimer Form for mislabelled/Unlabelled Samples*, available from Q-Pulse, must be signed and completed in addition to an incident form.

### **Sub-optimal specimens and rejection of samples**

Many samples sent for Molecular genetic or cytogenetic testing will be material that cannot be re-sampled (e.g. bone marrow, tumour biopsies). For this reason, sub-optimal specimens, for example aged, clotted or haemodilute specimens, will not be rejected and every effort will be made to obtain a result. However, it should be noted that failure rates are higher for these types of specimen and results from any failed specimens will include a description of the sample quality in the report.

All bone marrow samples will be processed (culture set up and/or nucleic acid extracted) as the patient has undergone a surgical procedure to obtain the sample, and this is not easily repeatable.

The majority of high-risk samples can be processed in the laboratory, and in practice, these are usually not rejected. Refer to RGP HAS0006: Health and Safety Manual. Please contact senior laboratory staff for advice.

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If a sample may have to be rejected, the duty scientist will liaise with a Senior Scientist or Head of Section, contact the sender to inform them that the sample will not be set up and, where necessary, arrange for a resample. Any rejected samples should be booked-in and a full report written explaining why the sample has been rejected

Samples sent to SIHMDS-AG in error may be returned to the sender. Contact the referring lab/clinician and enquire whether to dispose of or return the sample. The duty scientist will liaise with the sender, who, where necessary, will arrange for a courier pick-up. The sample should be re-packaged securely and booked-in EPIC with a note that sample was received in error with details of when and to whom the sample was returned.

### **Accepted Referrals**

#### **Haematological Malignancy Samples**

All paediatric haematological referrals on the National Genomic Test Directory for cancer are accepted for Cytogenetic and/or Molecular Genetics: RNA fusion panel is the default test for detection of fusion genes; FISH is only available for tests that are required for urgent treatment decisions or those where the result cannot be achieved in any other way.

Bone marrow is the preferred sample type. Peripheral blood specimens will be accepted at diagnosis or relapse if it is the appropriate sample type for the referral reason, or, if no bone marrow is available and there are sufficient numbers of circulating blasts in the patient's peripheral blood.

- Post-treatment remission samples will be accepted if a prior cytogenetic abnormality has been detected.
- Additionally, pleural fluid or cerebro-spinal fluid diagnostic and relapse specimens may be accepted.
- Infiltrating tumours

The main haemato-oncology investigations accepted by SIHMDS-AG include:

#### **Haemato-oncology:**

##### Acute Lymphoblastic leukaemia (ALL)

###### **B-ALL**

- Rapid FISH for *KMT2A* rearrangement, *BCR::ABL1* fusion, *MYC* rearrangement
- SNP array
- RNA fusion panel
- NGS panel

###### **T-ALL**

- RNA fusion panel
- NGS panel

##### Acute Myeloid Leukaemia (AML)

- FISH for *KMT2A* rearrangement, *TP53* deletion, *PML::RARA* fusion (if indicated)
- Karyotype
- RNA fusion panel
- NGS panel

##### Transient abnormal Myelopoiesis (TAM)

- NGS panel (*GATA1* gene variants)

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Myeloid leukaemia with Down Syndrome (ML-DS)

- Karyotype
- NGS panel

Burkitt Lymphoma/Leukaemia, Diffuse Large B-cell Lymphoma (DLBCL), B-Non-Hodgkins Lymphoma (B-NHL)

- Rapid FISH for *MYC*, *BCL2* and *BCL6* rearrangements, 11q deletion/duplication (if indicated)
- NGS panel

Chronic Myeloid Leukaemia (CML)

- Rapid FISH for *BCR::ABL1* fusion
- Karyotype
- RNA fusion panel
- NGS panel
- MRD testing for *BCR::ABL1* fusion transcript is performed at Imperial (Hammersmith Hospital)

Myeloproliferative Neoplasms (MPN)

- FISH for *BCR::ABL1* fusion
- Hyper eosinophilia syndrome: FISH for *FIP1L1::PDGFRA*, *PDGFRB* and *FGFR1*
- Karyotype
- RNA fusion panel
- NGS panel

Myelodysplastic syndromes (MDS) \*Blood samples are not appropriate for the assessment of MDS.

- Karyotype (MDS FISH panel for monosomy 5/7, deleted 5q/7q/20q, *MECOM* rearrangement if karyotype fails)
- RNA fusion panel
- NGS panel

Juvenile myelomonocytic leukemia (JMML)

- FISH for *BCR::ABL1* fusion
- Karyotype (FISH for monosomy 7 if karyotype fails)
- NGS panel

Bone marrow failure (BMF)

**Aplastic anaemia and neutropenia**

- FISH for monosomy 5/7, deleted 5q/7q
- Karyotype
- NGS panel

**Fanconi anaemia**

- FISH for monosomy 1q, 3q and 7q
- Karyotype (bone marrow samples only)

Other BMF referrals may be sent for the BMF NGS panel at King's College Hospital.

Langerhans Cell Histiocytosis (LCH)

- NGS panel

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Post-bone marrow transplant (BMT) chimerism and maternal engraftment

- FISH for chromosomes X and Y

**All other paediatric haemato-oncology referrals**

NGS panel and/or RNA fusion panel as indicated on the National Genomic Test Directory

**Solid Tumour Samples**

All paediatric solid tumour referrals on the National Genomic Test Directory for cancer are accepted for Cytogenetic and/or Molecular Genetics: Most solid tumour referrals received are sent for paediatric NGS panel and if requested, RNA fusion panel and methylation array. RNA fusion panel is the default test for the detection of fusion genes. FISH is only available for tests that are required for urgent treatment decisions or those where the result cannot be achieved in any other way.

The main solid tumour referrals accepted for investigation by SIHMDS-AG include:

- a) Diagnostic paediatric tumours
- b) Relapsed paediatric tumours

Sarcoma

- RNA fusion panel
- FISH testing may be performed in exceptional and/or urgent cases:

Rhabdomyosarcoma	<i>FOXO1</i> rearrangement
Ewings (PNET)	<i>EWSR1</i> rearrangement
Synovial Sarcoma	<i>SS18 (SYT)</i> rearrangement
Infantile Fibrosarcoma/ Congenital Mesoblastic Nephroma	<i>ETV6</i> rearrangement
Dermatofibrosarcoma protuberans (DFSP)	<i>COL1A1-PDGFB</i>

Medulloblastoma

- Rapid FISH for *MYCN* and *MYC* amplification
- NGS panel
- Methylation array

Neuroblastoma (NBL)

- Rapid FISH for *MYCN* amplification, 1p/q, 11q, 17q imbalance, *TERT* rearrangement
- SNP array
- NGS panel

Central Nervous System (CNS) tumours

- FISH for 1p/q and 19p/q (Oligodendroglioma only)
- RNA fusion panel
- NGS panel
- Methylation array

Refer to the National Genomic Test Directory for cancer which specifies the genomic tests commissioned by the NHS in England for cancer.

<https://www.england.nhs.uk/publication/national-genomic-test-directories/>

*The list of genetic tests provided by SIHMDS-AG is continually evolving. Please see website, North Thames Genomic Laboratory Hub – Bringing genomic research and testing together ([norththamesglh.nhs.uk](http://norththamesglh.nhs.uk))*

**Please contact the laboratory directly for any enquiries regarding testing.**

**NHS NORTH THAMES GENOMIC LABORATORY HUB  
GREAT ORMOND STREET HOSPITAL FOR CHILDREN NHS FOUNDATION TRUST  
LONDON**

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**Send away tests**

Send away tests	Clinical indications	External referral centre	Comments
<i>BCR::ABL</i> MRD	M91.9 BCR-ABL ALL sendaway M84.2 BCR-ABL: CML sendaway	Imperial college, Hammersmith Hospital	<i>BCR::ABL1</i> fusion transcript Analytical Report received
AML non-trial MRD	M80.14 AML MRD other qPCR	Dr Richard Dhillon Guy's Hospital	Fusion transcript Analytical Report received
<i>BRAF</i> <i>KIT</i>	M117.19 High sensitivity BRAF (LCH) M86.2 KIT D816 QF-PCR (SM/CM)	Royal Marsden	Analytical Report received
BMF panel	Acute Bone marrow failure (awaiting BMT)	Kings Royal Marsden	King's panel Send via GOSH RIDL. Analytical Report received (King's only)
	Non-urgent BMF	Kings	
NGS/RNA fusion panel	Haemato-oncology Solid tumour	Royal Marsden	Data received. Analysis of data performed at GOSH.

**Tests offered and associated turnaround times**

Please contact the laboratory for turnaround times (TAT) for specific tests.

Priority	Test	TAT
Very Urgent	Preliminary FISH for diagnostic acute leukaemia, Burkitt leukaemia and CML	3 days
Urgent	Diagnostic testing for acute leukaemia <b>only</b> : <ul style="list-style-type: none"> <li>• FISH</li> <li>• Karyotype (for Acute Myeloid Leukaemia only)</li> <li>• SNP array (for B-cell Acute Lymphoblastic Leukaemia poor risk genetics only)</li> </ul>	14 days
Routine	Testing for all other haemato-oncology and solid tumour referrals: <ul style="list-style-type: none"> <li>• FISH</li> <li>• Karyotype</li> <li>• SNP array</li> <li>• RNA fusion panel</li> <li>• NGS panel</li> <li>• Methylation array</li> </ul>	21 days

**Measurement of Uncertainty (MUC)**

Measurements of Uncertainty (MUC) values are not routinely included in reports, but the values are available upon request. MUC is used to indicate the confidence that the reported value is correct.