



National Centre for **Medical Genetics**
Ionad Náisiúnta **Gineolaíocht Leighis**

Division of Cytogenetics User Manual 2012

**This manual should be read in conjunction with
any service restrictions that may apply.**

**Division of Cytogenetics
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INTRODUCTION

Services:

The Division of Cytogenetics at the National Centre for Medical Genetics (NCMG) was established in January 1995 to provide a general cytogenetics service to the Irish population. It is housed at Our Lady's Children's Hospital, Crumlin (OLCHC), Dublin 12. The Laboratory operates in close collaboration with the Clinical Genetics Unit and the Division of Molecular Genetics at the NCMG. The website www.genetics.ie gives a comprehensive outline of the related disciplines within the Centre.

Cytogenetic testing involves analysing human chromosomes by karyotyping or other techniques, primarily at the microscope level. This valuable test allows detection of chromosome abnormalities which may cause congenital syndromes such as Down syndrome, serious malformation syndromes, developmental delay, infertility and recurrent pregnancy loss, and acquired malignancy disease such as leukaemia and lymphoma.

This service includes cytogenetic investigation of:

- **Constitutional cytogenetics**
 - blood samples
 - prenatal amniotic fluid and chorionic villus samples
 - analysis of pregnancy loss
 - solid tissue, e.g. skin for potential mosaicism or biochemical studies
- **Acquired abnormalities in haematology / oncology**
 - bone marrow samples in leukaemia and related disorders
 - lymph nodes for diagnosis of lymphoma
 - Infiltrated pleural fluid and ascites
 - Paediatric solid tumour biopsies, tumour touch preps, buccal smears, etc.
- **Molecular cytogenetics**
 - FISH on cultured and uncultured suspensions
 - FISH on cell preparations e.g. bone marrow smears

To achieve this, the Division has an establishment of 31 whole time equivalents (WTE) comprising scientific, technical and clerical staff. Clinical advice and interpretation of cytogenetic anomalies is readily available to referring clinicians either from Division of Cytogenetics staff or from the Division of Clinical Genetics, see Useful Contact Numbers. The Division participates in the United Kingdom National External Quality Assessment Scheme (UK NEQAS) in Clinical Cytogenetics and is accredited by Clinical Pathology Accreditation UK Ltd (CPA), Laboratory Reference Number 3002.

Occasionally specimens may require referral to other laboratories for testing. Where possible, the Division of Cytogenetics uses accredited laboratories; the current list is as follows:

Chromosome breakage syndromes:	Southmead Hospital, Bristol (Fanconi anaemia) University of Birmingham (Ataxia)
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Microarray analysis:

telangiectasia)
Western Regional Hospital, Edinburgh
Wessex Regional Genetics Lab,
Salisbury
Raboud University, Nimegen, Holland
Guy's Hospital, London

Due to ongoing staffing restrictions the Division may operate a restricted service for some referrals. Updates on the current status of restrictions can be found at www.genetics.ie/cytogenetics

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CONTACTING THE DIVISION OF CYTOGENETICS

Laboratory Hours Monday-Friday 09.30-17.00

Contacts within the Division of Cytogenetics are:

Cytogenetic Contacts:

David Betts (Chief Scientist)	409 6738
Adam Dunlop (Principal Scientist Constitutional & Quality Manager)	428 2704
Thomas Morris (Principal Scientist Molecular Cytogenetics)	428 2898
Johanna Kelly (Senior Scientist Haematology / Oncology)	428 2772
Aiveen Carey (Senior Scientist Prenatal Diagnosis)	428 2771
General enquiries	409 6737/6840
Specimen reception	428 2769
FAX	409 6971
Cytogenetics Laboratory	cytolab@olchc.ie
Web	www.genetics.ie

Other useful numbers and e-mail addresses within the National Centre for Medical Genetics are given at the end of this document, see Useful Contact Numbers.

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SENDING SAMPLES TO THE LABORATORY:

These notes apply to all sample types and should be consulted in conjunction with the sample categories listed below.

Diagnostic samples, now classified by the United Nations (UN) as Dangerous Goods, Division 6.2 and assigned to UN 3373, must be packaged for transport in a way that meets the requirements of Packaging Instruction 650. Such packaging may be specially purchased for this purpose or constructed from suitable components.

Packaging Instruction 650

Packaging should be strong enough to withstand the shocks and loadings normally encountered during transport, including manual and mechanical handling, and should be constructed and closed so as to prevent any loss of contents in the event of leakage or breakage. The packaging consists of:

1. **Primary receptacle**, leakproof and sealed, containing the specimen (e.g. Universal container or blood tube), not exceeding 50ml or 50g, individually wrapped with enough absorbent material to absorb all fluid in the event of leakage or breakage.
2. **Secondary packaging**, durable and leakproof container, to enclose and protect primary receptacle(s). Multiple individually wrapped primary receptacles may be placed in one secondary packaging. Sufficient absorbent material must be used to cushion multiple primary receptacles and absorb the entire contents of the primary receptacles in the event of leakage or breakage.
3. **Outer packaging** to protect the secondary packaging and contents from outside influences, such as physical damage and water while in transit.

In addition, the following **local rules** apply:

All samples should be in a sealed container accompanied by a fully completed NCMG request form. Packaging instructions are available on the website http://www.genetics.ie/pir/sending_samples.pdf. The following information must be legibly supplied with each sample, both on the form and the tube. The full sample identification policy is available on the website

<http://www.genetics.ie/pir/SampleIdentificationPolicyWeb.pdf>

- **Patient details**
 - full name
 - date of birth
 - hospital/medical record number
- **Referral Details**
 - sample type
 - date and time of sampling
 - sampling clinician including contact number
 - clinical indication
 - Stage of disease for haematology/oncology referrals (i.e. diagnosis, monitoring, relapse, post-BMT, etc)

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- tests required
- family history and any previous genetic studies on patient or family
- LMP/gestation (for prenatal samples)
- **Delivery of specimens**
 - Specimens should be delivered as soon as possible after sampling

Important note: samples without the above information may be rejected. Samples may also be rejected for other reasons, see individual sample types below.

Please mark **HIGH RISK SAMPLES** appropriately

- Forms and bottles must be labelled with a red warning sticker
- The sample must be sealed in a plastic bag. The form must never come into contact with the specimen tube or sample

Request forms can be ordered from the laboratory or downloaded from the website
Constitutional/Prenatal:

http://www.genetics.ie/pir/2006_NCMG_Referral_Form.pdf

Oncology/Haematology:

http://www.genetics.ie/documents/2010_NCMG_Onco_Referral_Form.pdf

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POSTNATAL CYTOGENETICS

Blood samples:

Constitutional karyotype analysis is performed on venous blood samples (5ml). Chromosomes are prepared from cultured blood lymphocytes.

The **main referral categories** are:

Mental retardation

Dysmorphism and congenital abnormality

Recurrent (3 or more) pregnancy losses

Infertility

Problems of sexual development

Requests for FISH studies (see page 9)

Please note that acceptance restrictions may apply, see www.genetics.ie

Reporting times:

Unfortunately due to staffing restrictions, reporting times may exceed international best practice guidelines (see website <http://www.genetics.ie/cytogenetics/>). Urgent samples are usually reported within 10 days.

For molecular genetic studies or DNA extraction, an EDTA sample is required (see Molecular Genetics User Manual or <http://www.genetics.ie/molecular/>). Consult the Division if in doubt.

Sample requirements:

- 5ml whole blood in lithium heparin (orange or dark green tops) well-mixed to prevent clotting (smaller samples are acceptable from infants)



- prompt dispatch to be received on the day of sampling, or the following day
- Sealed in a biohazard bag
- With a fully completed referral form in the outer pocket
- Samples sent via postal system must be packaged according to international guidelines, see packaging instructions on the website http://www.genetics.ie/pir/sending_samples.pdf

Suboptimal samples:

- Samples in an incorrect tube or clotted are unlikely to yield a result
- If prompt dispatch to the laboratory is not possible, samples should be kept at room temperature
- Samples delayed in transit or stored in suboptimal conditions may yield a substandard result and require repeat sampling
- Samples of 1-2ml are acceptable from neonates and cord blood
- For samples requiring specialist testing, e.g. Fanconi anaemia, please contact the laboratory before sampling

Limitations:

Conventional cytogenetics will not detect chromosome abnormalities beyond the resolution of the light microscope. Depending on the clinical indication, molecular cytogenetics (FISH) may be performed on these samples (see page 9).

For solid tissue samples on pregnancy loss, please see prenatal cytogenetics below.

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PRENATAL CYTOGENETICS

Prenatal detection of chromosome abnormalities by fetal karyotyping at either amniocentesis or chorionic villus sampling. Rapid dispatch to the Division of Cytogenetics is essential to a successful and speedy result.

Amniocentesis sampling:

An amniotic fluid sample (~15ml) is taken under ultrasound guidance, routinely at ~15-20 weeks gestation. Cells are cultured and karyotyped.

The **main referral categories** are:

- Maternal anxiety on the grounds of maternal age
- Increased risk of trisomy identified by maternal serum screening
- Abnormal ultrasound findings
- Family history of a chromosome abnormality
- Cell culture for biochemical/DNA analysis in single gene disorders

Please liaise with the laboratory on prenatal testing for other conditions

Reporting times:

- Reports are usually available within 14-21 days

Limitations:

Please note fetal karyotyping cannot necessarily detect subtle chromosome abnormalities or tissue specific mosaicism.

Sample requirements:

- Fresh amniotic fluid sample (~15ml in sterile labelled tube)
- Additional fluid may be required if molecular genetics studies are requested in addition to routine cytogenetics
- Sealed in a specimen bag
- With a fully completed NCMG referral form in the outer pocket
- Samples must be dispatched as soon as possible after collection
- Gestational age and any relevant obstetric details or scan findings should be noted

Suboptimal Samples:

The following samples may be unsuitable for chromosome analysis or may yield substandard results. They may also have slightly longer reporting times

- Small volume (<15ml)
- Significant blood staining
- Significant maternal cell contamination
- Inappropriately stored or transported samples
- Samples of late gestational age

Obstetricians will be notified of suboptimal samples by letter as soon as possible after receipt. It is the policy of NCMG to attempt cytogenetics on all suboptimal samples. The referring clinician will be informed within 10 days if a prenatal specimen shows no growth.

Rapid Aneuploidy Screen (QF-PCR):

Allows the rapid detection of Down syndrome, Patau syndrome, Edwards syndrome on uncultured amniocytes. Unfortunately this test is not performed at the NCMG and obstetricians should make their own arrangements for testing at an alternative laboratory.

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Chorionic Villus Sampling (CVS)

A small sample of placental villi taken trans-abdominally under ultrasound guidance, CVS can be performed earlier in pregnancy than amniocentesis, typically at 10-12 weeks.

The **main referral categories** are:

- Scan abnormalities
- Family history of chromosome abnormality
- Prenatal diagnosis of a molecular disorder, e.g. muscular dystrophy

CVS sampling is useful in cases where a scan abnormality is detected but the pregnancy is not far enough advanced for amniocentesis, or in cases of a previously known family history of a chromosomal or other genetic condition. Please note that fetal karyotyping may not necessarily detect subtle chromosome abnormalities or mosaicism.

Reporting Times:

- Reports are usually available within 14-21 days. Patients should be counselled accordingly
- Direct cultures which yield a quicker result may be employed in some instances, however these will not be reported upon until the full result is available. Patients should be counselled accordingly.

Sample requirements:

- Fresh CVS sample of approximately 20mg in a sterile conical tube containing sterile CVS transport medium
- Sterile CVS collection medium containing heparin is available on request from the laboratory and should be pre-warmed to room temperature before use
- Additional villi may be necessary if molecular studies are requested in addition to routine cytogenetics
- CVS collection medium is for *in vitro* use only
- Samples should be fully labelled, and accompanied by a fully completed NCMG request form
- Samples must be dispatched as soon as possible after collection

Suboptimal samples:

- Samples containing insufficient identifiable placental villi i.e. containing fewer than 4 chorionic villus branches
- Smaller samples (<20mg) may not achieve a result
- Some ~1-2% of CVS samples may be complicated by mosaicism, which could require a follow-up amniocentesis sample to resolve
- Samples for molecular analysis require larger sample sizes

Obstetricians will be notified of suboptimal samples by letter as soon as possible after receipt. It is the policy of NCMG to attempt cytogenetics on all suboptimal samples. The referring clinician will be informed within 10 days if a prenatal specimen shows no growth.

Limitations:

There may be discrepancies between the direct and long term karyotypes due to placental mosaicism, and patients should be counselled accordingly.

Please note that fetal karyotyping may not necessarily detect subtle chromosome abnormalities or mosaicism.

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For technical reasons high-risk samples may be more prone to complications of maternal cell contamination. In these cases it may be a better option to consider an amniocentesis sampling.

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SOLID TISSUE SAMPLES (INCLUDING PREGNANCY LOSS)

The main referral categories are:

- Investigating cases of pregnancy loss (where there are dysmorphic features or congenital abnormalities)
- Fetal abnormality detected by prenatal ultrasound
- Follow-up confirmation of prenatal findings
- Mosaicism studies of skin in patients with normal blood chromosomes, when diagnosis is problematic
- Culturing cells for molecular or biochemical investigations

Sample requirements:

Sterile tissue transport medium available on request. Please contact the laboratory

All samples should be accompanied by a fully completed NCMG request form

Failure to give adequate clinical information may result in the sample being discarded or inappropriate investigations being undertaken

If prompt dispatch to the Centre is not possible, specimens should be refrigerated at 4°C

Fetal samples

- A sample of fetal tissue (e.g. skin, muscle, etc)
- A sample of placenta 1cm³ taken from an area near the umbilical cord
- Fetal skin should be full depth and not a skin peel. Macerated samples are NOT suitable for culturing. For earlier losses, a sample of the products of conception may be sent

Skin Biopsies

- Skin samples should be 1cm³ and full depth.
- Some antiseptic creams may be detrimental to culture growth. A suggested method is to swab with alcohol or chlorohexidine and inject lignocaine intradermally

Suboptimal samples:

- Solid tissue samples are prone to microbial infection, which will result in culture failure
- Tissue should be kept in clean conditions, and handled by sterile instruments if possible
- Transport medium should not be retained beyond its expiry date, or results may be compromised
- **FRESH samples** only should be sent; formalin fixed specimens are unsuitable

Reports:

Reports are usually available within 28 days

Limitations

- Chromosome analysis from pregnancy loss may not detect subtle abnormalities
- Macerated samples will result in sample failure

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- Formalin fixed samples will not grow in culture and therefore will not yield a result
- For technical reasons high-risk samples are more prone to complications of maternal cell contamination

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HAEMATOLOGY/ONCOLOGY CYTOGENETICS:

Chromosome analysis in leukaemia and related disorders allows the detection of chromosomal and genetic changes acquired during the disease process. We provide a routine service for leukaemia and related disorders; some types of lymphoma, and paediatric and adolescent solid tumours, supplying information for diagnosis, prognosis and disease management.

Bone Marrow is the preferred sample in the analysis of leukaemia and related disorders (e.g. MDS), while for lymphoma; a fresh lymph node biopsy is required unless the bone marrow is infiltrated (ideally >20%). Leukaemic samples are triaged according to their urgency. Peripheral blood samples are acceptable in chronic conditions (e.g. CLL) or when bone marrow aspiration is difficult.

Main leukaemia related referral categories include:

Disease type:	G band performed:	FISH tests ¹ :	Sample types accepted:
ALL (B+T cell)	<ul style="list-style-type: none"> • Presentation • Follow up prior to 1st remission (if prev abnormality) • ?Relapse if prev. abn • Confirmed relapse 	BCR/ABL1, MLL, ETV6/RUNX1, TLX3, SIL-TAL1	<p>Bone marrow in RPMI with heparin</p> <p>An additional heparinised peripheral blood sample is also recommended when WBC >100</p>
AML	<ul style="list-style-type: none"> • Presentation • Follow up prior to 1st remission (if prev abnormality) • ?Relapse if prev. abnormality • Confirmed relapse 	MLL, BCR/ABL1, RUNX1/RUNX1T1, PML/RARA, CBFβ	Bone marrow in RPMI with heparin
CML	All samples	BCR/ABL1	<p>Bone marrow in RPMI with heparin.</p> <p>Peripheral blood is accepted for BCR/ABL1 FISH – only</p>
MDS	<ul style="list-style-type: none"> • Presentation • Follow up (on request only) • ?Relapse if prev. abnormality • Confirmed relapse 	Chromosome 5+7 status (only on request)	Bone marrow in RPMI with heparin

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MPN ²	<ul style="list-style-type: none"> • Presentation (disease subtype dependent) • Disease transformation 	BCR/ABL1 only on request	Bone marrow in RPMI with heparin
Eosinophilia	By Special request	FIP1L1-CHIC2-PDGFR4	Bone marrow or Peripheral blood in heparin
NHL ³	On request	IGH/CCND1, MYC, MYC/IGH, BCL6, ALK On request only IGH/BCL2, MALT	Lymph node biopsies or touch preps are preferred. Bone marrow in RPMI with heparin will be accepted only when BM infiltration is strongly suspected
Pre-transplant samples	As requested		Bone Marrow in RPMI with heparin
Chimerism	Not offered	CEP X/CEP Y Dual Colour	Bone marrow or Peripheral blood in heparin
Bone marrow failure disorders e.g SAA, SDS, etc	Presentation and Follow up		Bone marrow in RPMI with heparin
CLL	Presentation Follow up only in cases involving treatment related decision	ATM, TP53, 13q14, CEP 12, IGH/CCND1 as appropriate or on request only	Bone marrow or Peripheral blood in Heparin
Multiple Myeloma	Only FISH analysis performed at disease presentation, relapse cases will be considered if presentation FISH performed at this centre	Chromosome 1 status, TP53, IGH/FGFR3 On request 13q14/13q34, IGH/CCND1	Unstained BM smear slides with >15% plasma cells

¹ FISH tests employed may be dependent of disease subtype and/or G-banding results. Further FISH tests may be available on request.

² G-band analysis at presentation would be indicated on PMF, MDS/MPN cross over disease

³ Only MCL, BL, ALCL, DLBCL lymphomas will be routinely analysed. Other NHL related tests may be available on request

Please indicate if the patient has been entered into a trial

Solid tumours:

Paediatric and adolescent solid tumour samples are accepted. Please contact the laboratory for further information.

Sample requirements:

- Prompt (same day) dispatch to the laboratory is essential; delay may compromise results. To allow time to process the samples; they should arrive before 4pm on the day of aspiration.
- Bone marrow in heparinised transport medium
- Peripheral blood samples in lithium heparin (DARK GREEN or ORANGE) tubes (see illustration page 4) or heparinised transport medium.
- Lymph node biopsies and solid tumours should be sent in sterile transport medium
- If sending other infiltrated material such as ascites or pleural fluid it is recommended that heparin is added prior to submission.

Report times:

- Urgent bone marrows may have a preliminary FISH result within 4 days.
- Karyotypes for urgent bone marrows within 10 days.
- All samples are generally analysed in numerical order and turn around time may vary.
- In most cases confirmation of bone marrow morphology will be expected before any cytogenetic analysis is carried out. Please either contact the laboratory directly or fax a copy of the aspirate report to 01 4096971 as soon as possible to enable prompt processing of samples.

Limitations:

- Allowing samples to clot may result in sample failure.
- Delay in transit may compromise the ability to detect any abnormal clone present.
- Samples with low cellularity ($<6 \times 10^6/\text{ml}$) may not yield successful cultures.
- When cytogenetic analysis has not been performed at disease presentation; assessment of remission status cannot be performed. If no abnormality is present at disease presentation assessment of remission status cannot be performed.
- Optimal cytogenetic analysis on haematology/oncology specimens is obtained mainly through short term culture techniques. It is advisable that samples being sent on a Friday arrive before 12pm.
- Generally 25% of samples, on review of the morphology, do not require cytogenetic analysis. It is laboratory policy that morphology reports must be sent to the laboratory before analysis will be commenced.

MOLECULAR CYTOGENETICS (FISH):

Molecular cytogenetics (known as Fluorescence in situ Hybridization) can increase the speed, sensitivity and specificity of conventional cytogenetics. It can often be performed on the sample supplied for conventional cytogenetics, although sometimes a repeat sample will be required.

The technique takes advantage of a property of DNA where similar sequences are rendered single stranded, they will anneal, or “hybridise” together. By labelling probe sequences of interest with fluorochromes, we can visualise specific sequences on a slide of patient material, using image analysis software. There are a variety of FISH applications outlined below. Please contact the laboratory for details.

Microdeletion analysis:

Used in cases where the referring clinician suspects a specific syndrome. These are often not detectable by conventional cytogenetics. Syndromes where a FISH test is available include:

- 1p36 Microdeletion syndrome
- Wolf-Hirschhorn
- Cri-du-chat
- Sotos
- Williams
- Langer Giedion/TRPS
- Retinoblastoma
- Rubinstein–Taybi
- Miller-Dieker
- Smith-Magenis
- 22q11.2 deletion or duplication
- Phelan-McDermid
- X-Linked Ichthyosis
- Kallman
- This list is not exhaustive and more disorders may currently be investigated.

Note: Prader-Willi/Angelman (PWS/AS) Syndromes: Testing for PWS/AS are no longer routinely undertaken by FISH. All samples are referred to our Molecular Genetics laboratory for MLPA analyses.

Detection of Gene Rearrangement in cancer:

Neoplastic “fusion genes” may be created by rearrangement of specific genetic material. This is a recognised cause of many cancers and can be highly specific. By use of two- or three-colour FISH probes to both gene partners, the novel sequences may be identified by the close juxtaposition of signals as a fusion product (usually a cancer gene or promotor gene). The haematological neoplasms have been most extensively studied to date.

Sample requirements:

FISH studies can usually be carried out on the samples referred for conventional cytogenetics. Consult the individual sections above for advice.

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Reports:

Reports are usually available at the same time or before conventional cytogenetics. If the result is required urgently, they can be reported by telephone to the referring clinician.

Limitations:

The limitations of FISH tests are extremely variable, depending on the material tested, and the clinical context of the test. Individual reports give limitations if applicable. Please contact the laboratory if you wish to discuss the suitability of a particular test.

Note: **Subtelomere Screening:** This test is no longer routinely undertaken by FISH. All samples are referred to external laboratories for MLPA analyses.

IMPROVEMENTS TO THE SERVICE:

The Division of Cytogenetics operates a quality management system which includes regular review and audit. We aim to provide a high quality service and are continually improving our service. In order that we may do this efficiently, we value any constructive comments from our Users. If you have any comments you would like to make, please feel free to contact the Section Head or our Quality Manager.

Adam Dunlop ☎ 01 428 2704

✉: adam.dunlop@olchc.ie

COMPLAINTS:

All complaints should be brought to the attention of the Head of Division, Section Heads, or the Quality Manager. Contact numbers are given in pages 4 and 17.

Useful Contact Numbers:

Division of Cytogenetics

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Chief Scientist -Cytogenetics

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Please note that NCMG laboratory staff adhere to a strict telephone policy and **will not** take enquiries from patients. **All** patient contact must be directed via the referring clinician.

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