



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

The National Centre for Medical Genetics
Our Lady's Children's Hospital, Crumlin
Dublin 12

Division of Cytogenetics

User Guide 2014

Please note this User Manual must be read in conjunction with any service restrictions that may apply; see www.genetics.ie

This version released January 2014





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Introduction

The Division of Cytogenetics aims to provide a comprehensive and efficient analytical, training and educational service of the highest quality to its users within the geographical area of the Republic of Ireland.

This handbook summarises information on the services provided and should be read in conjunction with any service limitation and restrictions that may be in place (available on www.genetics.ie). The Division aims to return correct results, for the correct patient, to the correct place and person within the correct time frame. The quality of our service is regularly audited and was accredited by CPA (UK) Ltd until end of 2013 (Laboratory Number 3002). The Division is working towards Irish National Accreditation Board (INAB) accreditation to International Standard ISO 15189:2012.

The Division of Cytogenetics participates in external quality assurance (EQA) for all sample types. Current certificates from UK National External Quality Assessment Scheme (UK NEQAS) in Clinical Cytogenetics (CCNEQAS) are available on demand.

It is important that you contact us if you have any questions, comments or complaints about any aspect of our service. Contact details can be found below.

Division of Cytogenetics Location

The Division of Cytogenetics is housed within the National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

Please note there is no patient access to the laboratories.



Map of OLCHC site, National Centre for Medical Genetics shown in orange

Postal Address

The address for the Division of Cytogenetics is:



Division of Cytogenetics
National Centre for Medical Genetics
Our Lady's Children's Hospital
Crumlin
Dublin 12

Opening Hours

Weekdays 09.30 – 17.00 (excluding public holidays)

Deliveries to the Laboratories

Specimens can be delivered to the laboratories:

From within Our Lady's Children's Hospital - By delivery to the laboratory or by internal chute system (destination 2770).

From outside Our Lady's Children's Hospital - By delivery to the specimen reception located at the rear of the National Centre for Medical Genetics. Access is via Gate 5 at the rear of Our Lady's Children's Hospital, Crumlin.

See also page 20, Sending Samples to the Laboratory

Contact Information

General				
	Name	Telephone	Internal	email
Chief Scientist	David Betts	01 409 6738	6738	david.betts@olchc.ie
Admin/Enquiries	Lynn Devitt	01 409 6737	6737	lynn.devitt@olchc.ie cytolab@olchc.ie
Quality Manager	Adam Dunlop	01 428 2704	2704	adam.dunlop@olchc.ie
Constitutional Cytogenetics				
Team		Telephone	Internal	email
Prenatal Team		01 409 6735	6735	aiveen.carey@olchc.ie
Postnatal Team		01 409 6735	6735	annmarie.hegarty@olchc.ie
Haematology / Oncology				
Team		Telephone	Internal	email
Haematology/ Oncology Team		01 428 2772	2772	johanna.kelly@olchc.ie
Fluorescence In Situ Hybridisation (FISH)				
Team		Telephone	Internal	email
FISH Team		01 428 2898	2898	thomas.morris@olchc.ie
Microarray				
Team		Telephone	Internal	email
Microarray Team		01 428 2771	2771	jennifer.mcdaid@olchc.ie

Useful Links

Division of Cytogenetics Quality Manager

☎ 01 428 2704 (internal 2704) adam.dunlop@olchc.ie

Division of Molecular Genetics Enquiries

☎ 01 409 6733 (internal 6733) dnalab@olchc.ie

Division of Molecular Genetics Quality Manager (Christine Brady)

☎ 01 428 2899 (internal 2899) christine.brady@olchc.ie

Division of Clinical Genetics Enquiries

☎ 01 409 6739 (internal 6739)

National Centre for Medical Genetics General Manager

☎ 01 409 6277 (internal 6277) damien.moyles@olchc.ie

Cytogenetic Prenatal Tests

Contacts: Cytogenetic Administration ☎ 01 409 6737 (internal 6737)
Cytogenetic Laboratory ☎ 01 409 6735 (internal 6735)
✉ cytolab@olchc.ie

Prenatal diagnosis is offered to those couples that are at risk of having a chromosomally abnormal pregnancy.

At risk pregnancies are identified through one or more of the following factors:

- 'High Risk' maternal serum screening test result increasing the mother's risk of carrying a fetus with a chromosome abnormality
- Increased maternal age
- Positive family history (i.e. previously affected child or one parent known to be a carrier of a chromosome rearrangement)
- Anomalies seen on ultrasound scan indicative of a chromosome abnormality

Having identified the at-risk pregnancy prospective parents may have the option of whether or not to undergo a prenatal diagnostic test and choosing the most suitable type of prenatal test for them. The type of test may also be dependent on the gestation of the pregnancy. Guidance is available from the medical practitioner.

Prenatal detection of chromosome abnormalities by fetal karyotyping at either amniocentesis or chorionic villus sampling requires rapid dispatch to the Division of Cytogenetics in order for a successful and speedy result.

Amniocentesis

This procedure involves trans-abdominal needle sampling of the amniotic fluid; a 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.

Any specialist requests for cytogenetic testing, e.g. FISH, MUST be first discussed by telephone with the Prenatal Team (☎ 01 409 6735) or the Head of Cytogenetics before taking the specimen. Even after the discussion, all the information must be included on the referral form.

Reporting Times

Reports are usually available within 14 - 21 days. For current report times, please see our website www.genetics.ie. It should be noted that occasionally additional work is necessary

and delays may occur.

Limitations

Please note fetal karyotyping cannot necessarily detect subtle chromosome abnormalities or tissue specific mosaicism. See also Page 23.

Sample Requirements

- Fresh **amniotic fluid** sample (~15ml in sterile labeled tube)
- Additional fluid may be required if molecular genetics studies are requested in addition to routine cytogenetics
- Sealed in a specimen bag in accordance with packaging instructions, see Page 20
- 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
- All materials used in the collection of samples must be disposed of in accordance with local policies

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.

Chorionic Villus Sampling (CVS)

This procedure involves a trans-abdominal needle sampling of the placenta under ultrasound guidance. CVS can be performed earlier in pregnancy than amniocentesis, typically at 10-12 weeks. This procedure is performed by specialist Maternity Hospitals and advice is available from the medical practitioner.

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.

Any specialist requests for cytogenetic testing, e.g. FISH, **MUST** be first discussed by

telephone with the Prenatal Team (01 409 6735) or the Head of Cytogenetics before taking the specimen. Even after the discussion, all the information must be included on the referral form.

Reporting Times

Reports are usually available within 14 - 21 days. For current report times, please see our website www.genetics.ie. Patients should be counseled 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 counseled accordingly.

It should be noted that occasionally additional work is necessary and delays may occur.

Sample Requirements

- Fresh **chorionic villus** 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
- A larger chorionic villus sample 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 labeled, and accompanied by a fully completed NCMG request form and packaged in accordance with packaging instructions, see Page 20
- 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

Referring clinicians 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 sample shows no growth.

Limitations

There may be discrepancies between the direct and long term karyotypes due to placental mosaicism, and patients should be counseled accordingly. Please note that fetal karyotyping may not necessarily detect subtle chromosome abnormalities or mosaicism.

In these cases it may be a better option to consider an amniocentesis sampling.

For technical reasons high-risk samples may be more prone to complications of maternal cell contamination.

See also Page 23.

Requests for Additional Tests

Cell cultures are usually discarded once the cytogenetic report had been issued. Fixed cell

suspensions are usually discarded after around 6 months after the birth of the baby, therefore additional requests for testing, e.g. FISH, and may not be possible after that time period. Occasionally cells will be frozen in liquid nitrogen; this will be stated in the cytogenetic report.

User Responsibilities

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that microscope slides will be stored for a period of time. Where requested by a Consultant Clinical Geneticist, cells will be stored in a Liquid Nitrogen cell bank.
- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Informing us about cases that require specific urgent attention.

- 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.

Reporting Times

Reports are usually available within 28 days. It should be noted that occasionally additional work is necessary and delays may occur.

Limitations

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

See also Page 23.

Requests for Additional Tests

Cell suspensions are usually discarded after around 4 months, therefore additional requests for testing, e.g. FISH, and may not be possible after that time period. Occasionally cells will be frozen in liquid nitrogen; this will be stated in the cytogenetic report.

Send Out Tests

Certain diagnostic tests may be referred to other accredited laboratories where there is a greater expertise. In this instance both the report and invoice for testing will be sent by the referral lab to the clinician requesting the test. The Division of Cytogenetics does not transcribe reports.

Material may be sent out to other laboratories for research purposes at the request of a Consultant Clinical Geneticist.

User Responsibilities

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that microscope slides will be stored for a period of time. Where requested, cells derived from cultured tissue will be stored in a Liquid Nitrogen cell bank.
- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Informing us about cases that require specific urgent attention.

Cytogenetic Postnatal Tests

Contacts: Cytogenetics Administration ☎ 01 409 6737 (internal 6737)
Cytogenetics Laboratory ☎ 01 409 6735 (internal 6735)
✉ cytolab@olchc.ie

Blood Samples

Blood chromosome analysis (or karyotyping) is undertaken to identify constitutional abnormalities (abnormalities which have been present since conception). The abnormalities may be in the form of alterations in the structure of one or more chromosomes (chromosome rearrangements), or may consist of an entire extra or missing chromosome (aneuploidy). If the result of a rearrangement is the net gain or loss (duplication or deficiency) of chromosome material (an 'unbalanced karyotype') then phenotypic effects are likely. If the structural abnormality simply results in rearranged chromosome material (with no net duplication or deficiency) then the karyotype is termed 'balanced'. In such an instance adverse effects are unlikely although the individual carrying such a rearrangement may be predisposed to having children with a derivative 'unbalanced' chromosome constitution.

Cultures of lymphocytes are established which, following a preparatory period, allow the visualization of the chromosomes in cells which are undergoing cell division (mitosis).

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

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

Reporting Times

For current reporting times, please see our 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 Constitutional Team if in doubt ☎ 01 409 6841.

Sample Requirements

- ~5ml **whole blood** in lithium heparin (orange or dark green tops) well-mixed to prevent clotting (smaller samples are acceptable from infants).
- Please **DO NOT** use small tapered blood tubes as clotting tends to occur



- Prompt dispatch to be received on the day of sampling, or the following day.
- Sealed in a biohazard bag and packaged in accordance with packaging instructions, see Page 20, with a fully completed referral form in the outer pocket.
- All materials used in the collection of samples must be disposed of in accordance with local policies.

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 Constitutional Team (☎ 01 409 6735) before sampling as this may require it to be sent to another laboratory and may incur a cost, see Send Out Tests.

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 17).

For solid tissue samples on pregnancy loss, please see Cytogenetics Prenatal Tests above. See also Page 23.

Send Out Tests

Certain diagnostic tests may be referred to other accredited laboratories where there is a greater expertise. In this instance both the report and invoice for testing will be sent by the referral lab to the clinician requesting the test. The Division of Cytogenetics does not transcribe reports.

Fanconi Anaemia (OMM # 227650) referred to Bristol Genetics Laboratory (UK)

Ataxia Telangiectasia (OMIM #208900) referred to Bristol Genetics Laboratory (UK)

Bloom syndrome (OMIM #210900) referred to Bristol Genetics laboratory (UK)

Additional tests may be sent out at the request of a Consultant Clinical Geneticist.

Requests for Additional Tests

Cell suspensions are usually discarded after around 4 months, therefore additional requests for testing, e.g. FISH, and may not be possible after that time period.

User Responsibilities

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that microscope slides will be stored for a period of time (dependent on

sample type).

- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Informing us about cases that require specific urgent attention.

Disease type:	Please send the following sample type:
ALL (B+T cell) <ul style="list-style-type: none"> • Presentation • ?Relapse if prev. abnormality • Confirmed relapse 	Bone Marrow aspirate in RPMI with heparin An additional heparinised Peripheral Blood sample is also recommended when WBC >100
AML <ul style="list-style-type: none"> • Presentation • Follow up prior to 1st remission (if previous abnormality) • ? Relapse if previous abnormality • Confirmed relapse 	Bone Marrow aspirate in RPMI with heparin Peripheral Blood sample is recommended if dry tap and circulating blasts present
CML <ul style="list-style-type: none"> • Presentation • Follow up • Relapse 	Bone Marrow aspirate in RPMI with heparin. Peripheral Blood is accepted for BCR/ABL1 FISH – only at presentation (but should <u>not</u> be sent at follow up)
MDS (and MDS/MPN disease) <ul style="list-style-type: none"> • Presentation • Follow up (on request only) • ? Transformation • Confirmed relapse 	Bone Marrow aspirate in RPMI with heparin
?MDS <ul style="list-style-type: none"> • Presentation • Persistence of unexplained disease • Aplastic anaemia when possible transformation 	Bone Marrow aspirate in RPMI with heparin
MPN <ul style="list-style-type: none"> • Presentation (atypical ET and/or PMF) • Disease transformation 	Bone Marrow aspirate in RPMI with heparin Heparinised Peripheral Blood is acceptable for PMF
Eosinophilia <ul style="list-style-type: none"> • By special request 	Bone Marrow aspirate or Peripheral Blood in heparin
Lymphoma (adult) <ul style="list-style-type: none"> • Burkitt • DLBCL v Burkitt v Two hit • MCL 	Unstained Bone Marrow smear slides with confirmed infiltration (>3%) of the aspirate
Pre-transplant samples	Bone Marrow aspirate in RPMI with heparin
Bone marrow failure disorders e.g. DBA, SDS, etc <ul style="list-style-type: none"> • Presentation and Follow up 	Bone Marrow aspirate in RPMI with heparin
CLL <ul style="list-style-type: none"> • Presentation • Differential diagnosis CLL v MCL • Follow up only in cases involving treatment related decision 	Bone Marrow aspirate or Peripheral Blood in heparin or EDTA
Multiple Myeloma <ul style="list-style-type: none"> • Presentation • Follow up only in cases involving treatment related decision 	Unstained Bone Marrow Smear slides with >15% plasma cells

Sample Requirements

Bone marrow	Heparinised transport medium. To ensure sufficient cells approx. 2ml of - bone marrow is recommended that is derived from the first or second pull, and packaged in accordance with packaging instructions, see Page 20.
Peripheral blood	In lithium heparin (DARK GREEN or ORANGE) tubes (see illustration page - 4) or heparinised transport medium and packaged in accordance with packaging instructions, see Page 20.
Lymph node biopsies	- Sterile transport medium and packaged in accordance with packaging instructions, see Page 20.
Solid tumours	- Sterile transport medium and packaged in accordance with packaging instructions, see Page 20.
Ascites or pleural fluid	It is recommended that heparin is added prior to submission if sending other - infiltrated material such as ascites or pleural fluid. All samples must be packaged in accordance with packaging instructions, see Page 20.
	<ul style="list-style-type: none">• 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.
	<ul style="list-style-type: none">• All materials used in the collection of samples must be disposed of in accordance with local policies.
	<ul style="list-style-type: none">• Please indicate if the patient has been entered into a trial.

Report Times

- Urgent referrals may have a preliminary FISH result within 4 days
- Karyotypes for urgent referrals within 10 days All cases are generally analysed in numerical order and turn around time may vary.
- In most cases confirmation of morphology will be expected before any cytogenetic analysis is carried out. Please either contact the Haematology/Oncology Team directly on 01 428 2772 or fax a copy of the aspirate report to 01 409 6971 as soon as possible to enable prompt processing of samples.

It should be noted that occasionally additional work is necessary and delays may occur.

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 ($<10 \times 10^6$ nucleated cells) may not yield successful cultures.
- Bone marrow samples that derive from the '3rd pull' may not contain sufficient disease cells to obtain representative cytogenetic result.
- When cytogenetic analysis has not been performed or 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 (FAX: 01 409 6971).
See also Page 23.

Requesting Additional Tests

Cell suspensions are usually discarded after around 4 months, therefore additional requests for testing, e.g. FISH, and may not be possible after that time period.

User responsibilities

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that microscope slides will be stored for a number of years. Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Informing us about cases that require specific urgent attention.

Molecular Cytogenetics (FISH)

**Contact: Cytogenetic Administration
Cytogenetics Laboratory**

☎ 01 409 6737 (internal 6737)

☎ 01 409 6735 (internal 6735)

✉ cytolab@olchc.ie

Fluorescence in situ Hybridization (FISH) (sometimes referred to as Molecular cytogenetics) 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 labeling probe sequences of interest with fluorochromes, we can visualize specific sequences on a slide of patient material, using image analysis software. There are a variety of FISH applications outlined below. Please contact the FISH Team for details (☎ 01 409 6735).

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-Beuren
- Langer Giedion/TRPS
- Retinoblastoma
- Rubinstein–Taybi Miller-Dieker
- Smith-Magenis
- 22q11.2 deletion or duplication
- 22q13 deletion (Phelan-McDermid syndrome)
- 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 is 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 promoter gene). The haematological neoplasms have been most extensively studied to date.

Sample Requirements

Metaphase FISH studies can usually be carried out on the samples referred for conventional cytogenetics. Interphase FISH can be performed on a variety of slide preparations, including touch preps, buccal smears, cytopins, etc. Consult the individual teams above for advice. Samples must be submitted in accordance with packaging instructions, see Page 20. All materials used in the collection of samples must be disposed of in accordance with local policies.

Reporting

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 FISH Team (☎ 01 409 6735) if you wish to discuss the suitability of a particular test. See also Page 23.

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

Requesting Additional Tests

User Responsibility

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that material may be stored for a period of time determined by the material type.
- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.
- Informing us about cases that require specific urgent attention.

Microarray

**Contact: Cytogenetics Administration
Microarray Laboratory**

**☎ 01 409 6737 (internal 6737)
☎ 01 428 2771 (internal 2771)
✉ cytolab@olchc.ie**

Array-based comparative genomic hybridization (array CGH), also called microarray analysis, is a cytogenetic technology that evaluates areas of the human genome for gains or losses of chromosome segments at a higher resolution than traditional karyotyping. This technology can dramatically improve the resolution and clinical utility of cytogenetic analyses in a range of clinical settings. Array CGH is performed on high quality DNA extracted from whole blood in EDTA. Array CGH is only available by service level agreement (see below). Array CGH can detect microscopic and submicroscopic deletions and duplications across the genome, including loci of known microdeletion/microduplication syndromes, subtelomeric regions, and pericentromeric regions. Array CGH will also identify marker chromosomes, some cases of mosaicism, and aneuploidy.

The main referral categories are:

- Clinically significant abnormal growth - short stature, excessive growth, microcephaly, macrocephaly
- Abnormal clinical phenotype or dysmorphism
- Multiple congenital abnormalities
- Mental retardation or developmental delay
- Suspected deletion / microdeletion / duplication syndrome
- X-linked recessive disorder in a female

Currently the laboratory only offers a postnatal peripheral blood microarray service. Testing for other tissue types will be added as and when resources allow. Users will be notified should the range of sample types accepted increases.

Service level Agreements

The Division has established service level agreements with a number of institutions to provide a microarray service. There is a charge for this service, please contact the Microarray Team (☎ 01 428 2771) for details. The service will be expanded as and when funding and infrastructure allows.

Sample Requirements

- 5ml whole blood in EDTA (red or purple tops) well-mixed to prevent clotting (smaller samples are acceptable from infants) – fasting is not necessary
- 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, available from www.genetics.ie
- Please avoid using tapered blood tubes as clotting tends to occur

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 4°C.

- Samples stored in suboptimal conditions may yield a substandard result and require repeat sampling.

Reporting Times

Reports are usually available within 28 days. For current report times, please see our website www.genetics.ie

Limitations

Array CGH will not detect:

- Balanced chromosomal rearrangements
- Single gene disorders
- Ploidy abnormalities
- Low level mosaicism (below 30%)
- Epigenetic anomalies
- Alterations in chromosome structure at areas of the genome not covered by the array platform.

We currently report on imbalances at 300kb for gains and 100kb loss resolution. Interpretation of aCGH analysis is based on current knowledge of the genome.

Requesting Additional Tests

Original blood tubes are stored for 2 months in the Division of Cytogenetics. DNA is stored indefinitely in the Division of Cytogenetics. If additional DNA based tests are required, please contact DNALAB@olchc.ie

User Responsibility

Delivery of our cytogenetics service is dependent on the co-operation of the user. The user is responsible for:

- Obtaining appropriate consent from the patient for the required test and making the patient aware that material may be stored for a period of time determined by the material type.
- Despatching samples in a timely manner to arrive on a weekday within normal working hours of between 9.30am and 5.00pm.
- Ensuring that all samples are accompanied by a **request form** or covering letter with full details including which specific tests are required.

Informing us about cases that require specific urgent attention.

Sending Samples to the Laboratory

The referring facility must inform the patient of any preparatory requirements prior to sampling and of the consequences and implications of genetic testing. All materials used in the collection of samples must be disposed of in accordance with local policies.

The following packaging 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

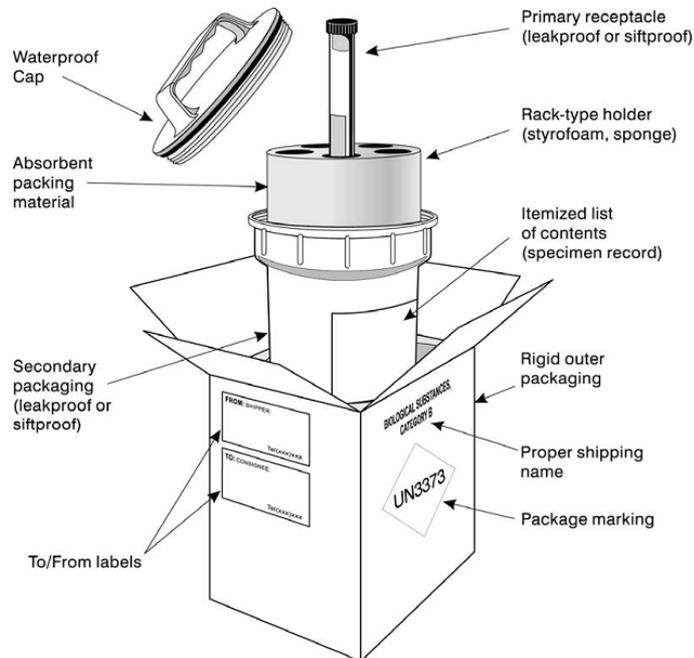
Packaging should be strong enough to withstand the shocks and loadings normally encountered during transport, including manual and mechanical handling, and should be constructed and closed so as to prevent any loss of contents in the event of leakage or breakage. The packaging consists of:

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>



Delivery of Specimens

Specimens should be delivered as soon as possible after sampling. Transport requirements for each sample type are outlined above and on the reverse of the NCMG referral form.

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

High Risk Samples

- Please mark **HIGH RISK SAMPLES** appropriately.
- Forms and bottles must be labeled 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.

The Cytogenetics laboratory cannot accept samples from patients who have or are suspected of having Group 3 or 4 pathogens.

Request Forms

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

Constitutional/Prenatal/Microarray:

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

Oncology/Haematology:

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

Details Required on Request Form

- **Patient details**
 - full name
 - date of birth
 - hospital/medical record number
- **Referral Details**
 - sample type
 - date and time of sampling
 - name of person taking the sample
 - requesting clinician including contact number
 - clinical indication
 - stage of disease for haematology/oncology referrals (i.e. diagnosis, monitoring, relapse, post-BMT, etc)
 - tests required
 - family history and any previous genetic studies on patient or family (see Data protection and confidentiality below)
 - LMP/gestation (for prenatal samples)

Data Protection and Confidentiality

The National Centre for Medical Genetics adheres to the Data Protection Act 1998 and 2003; Our Lady's Children's Hospital, Crumlin has a Data Control Officer based in the Information Technology Department.

The National Centre for Medical Genetics is legally bound by the Freedom of Information Act 1997 and 2003; Our Lady's Children's Hospital, Crumlin has a Freedom of Information Officer located within the Patient Support Unit.

Private Hospitals

Samples from private hospitals must adhere to our acceptance criteria and must have signed an agreement of payment for testing. For further information please contact the General Manager (Damien Moyles) on ☎ 01 409 6277 or Chief Scientist, David Betts on ☎ 01 409 6738.

Service Level Agreements for Microarray Analysis

Microarray analysis is performed according to service level agreements. The Division is working towards extend this service to all hospitals as funding allows. For further information please contact the Chief Scientist david.betts@olchc.ie .

Limitations of Cytogenetic Investigations

Failed Samples

Karyotypic investigations require the culture of living cells and therefore delays in transport of the sample or exposure to temperatures above 38°C or below 0°C will result in the sample failing to give a result.

General Cytogenetic Analysis (Karyotype)

Analysis by karyotype is the visual inspection of a series of stained bands along the length of each chromosome. Generally blood samples give the best quality chromosomes and therefore provide the best chance of detecting small subtle chromosome abnormalities.

Chromosomes from other tissues (e.g. amniotic fluid, chorionic villus, skin, and bone marrow) are generally shorter and have less banding detail; this therefore increases the risk of missing a subtle chromosome abnormality. Some cytogenetic abnormalities may involve quite large segments of material being exchanged between chromosomes, however, due to similarity in their banding pattern; the resulting abnormality may remain cryptic.

Some chromosome abnormalities are beyond the limit of the light microscope.

In some cases parental blood sample may be required to help us interpret complex findings or to assess the implication of a cytogenetic result to the wider family.

Mosaicism

Cytogenetic mosaicism is the presence of more than one chromosomally different cell line. The likelihood of detecting mosaicism increases with the number of cells analysed. Generally, the karyotyping of a sample will involve looking at a relatively small number of cells (10 or less) and is not an effective way of detecting mosaicism. However, if there is an indication of suspected mosaicism, additional cells will be examined.

Prenatal Samples

In a small number of cases maternal, and occasionally paternal, blood will be required to interpret equivocal results, caused primarily by maternal cell contamination. Very rarely there will be a false negative in prenatal samples due to the presence of maternal cell contamination.

An abnormal chromosome complement may be present in only a proportion of cells from the foetus (mosaicism).

A chromosome abnormality may be present on a proportion of cells from one or more cultures initiated from the sample. This may be due to a cultural artefact (pseudomosaicism) or growth of extra-embryonic cells, but may be indicative of an abnormal cell line in the foetus.

Very rarely, growth of maternal cells in the sample may result in the analysis indicating a normal female karyotype which is not indicative of the foetal chromosome constitution.

Amniocentesis and chorionic villus sampling is a screening process for major chromosome abnormalities such as trisomy and monosomy. Although a number of less obvious abnormalities such as deletions, duplications and translocations are often detected, it is possible these rearrangements will not be detected.

Abnormalities such as fragile X or chromosome instability syndromes, which require specific culture conditions, will not be detected.

Microarray (aCGH)

See Section entitled Microarray

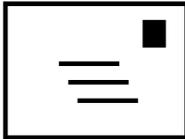
Complaints and Feedback

Your feedback is important to us. Any complaints, compliments or feedback may be directed to either the Quality Manager or Chief Scientist.

Adam Dunlop Principal Scientist & Quality Manager ☎ 01 428 2704 adam.dunlop@olchc.ie

David Betts Chief Scientist ☎ 01 409 6738 david.betts@olchc.ie

Or by mail to:



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Dublin 12

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