



CYTOGENETICS USER MANUAL 2008

(available as a pdf. Downloadable from the department website)

Laboratory Hours
Monday - Friday 9.00am-5.00pm

Website: www.humangenetics.org.uk

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CONTENTS:

INTRODUCTION:

- **Services** **page 2**
- **Contacts** **page 2**

GENERAL NOTES:

- **Completion of Request Forms** **page 3**
- **UKNEQAS Performance:** **page 3**

POSTNATAL:

- **Bloods** **page 4**

PRENATAL:

- **Amniocentesis** **page 5**
- **Chorionic villus** **page 6**
- **Solid Tissue (Pregnancy loss & TOP)** **page 7**

ONCOLOGY: **page 8**

MOLECULAR CYTOGENETICS:

- **FISH** **page 9**
- **HER 2 status** **page 10**
- **Array Comparative Genomic Hybridisation** **page 11**

RESEARCH AND DEVELOPMENT: **page 12**

INTRODUCTION

Services:

The department delivers a range of specialised diagnostic cytogenetic services to Tayside and North East Fife, together with the research and development necessary for new clinical services of direct patient benefit. Close liaison with Clinical and Molecular Genetics disciplines helps to provide an integrated Human Genetics Service. A User Survey is circulated every two years, but we welcome any User comments or suggestions. The www.humangenetics.org.uk homepage gives further information about the related disciplines within the unit

Contacts:

Cytogenetics Contacts:		
Dr. Norman Pratt	x32680	01382-632680
Miss. C. Maliszewska	x36735	01382-496735
General Enquiries	x36272	
Specimen Reception	x33107	
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Molecular Genetics Contacts:		
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Clinical Genetics Contacts:		
Dr. David Goudie	x31629	01382-632169

Cytogenetics: involves the analysis of human chromosome metaphase spreads in the form of a karyotype, to identify chromosome abnormalities that may be clinically significant. The repertoire of the department covers the areas of:

- constitutional cytogenetics
 - blood samples
 - prenatal amniocentesis & chorionic villus sampling
 - analysis of pregnancy loss and TOPs
 - solid tissue eg: skin for potential mosaicism or biochemical studies
- acquired abnormalities in Oncology
 - bone marrow samples in leukaemia and related disorders
 - lymph nodes for diagnosis of lymphoma
 - peripheral blood in chronic disorders
- molecular cytogenetics
 - FISH on cultured and uncultured suspensions
 - FISH on paraffin sections
 - Array comparative genomic hybridisation on extracted patient DNA

Chromosomal abnormality may play a role in a variety of disorders including: congenital syndromes such as Down syndrome; mental retardation or developmental delay; infertility & recurrent pregnancy loss; and acquired malignant disease including leukaemia and lymphoma.

Clinical advice and interpretation: is available from both clinical scientists (Dr. N. Pratt, Head of Laboratory Services and Miss C. Maliszewska, Principal Scientist) and Clinical Genetics staff (Dr. D Goudie and Dr. J. Berg). The website gives further details, while sample requirements and expected reporting times are summarised in the individual sections below.

GENERAL NOTES:

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

All samples should be in a sealable specimen bag accompanied by a Human Genetics request form and the following information must be supplied legibly with each sample both on the form and the sample tubes, where appropriate:

- patient details:
 - Surname
 - Prename
 - Date of birth & CHI number
 - Referring consultant

- referral details:
 - sample type
 - date and time of sampling
 - sampling clinician contact details and location
 - clinical condition
 - tests required
 - family history and any previous genetic studies in patient or family
 - LMP/gestation for prenatal samples

Important note: samples without the above information may be rejected. Samples may also be considered unacceptable for other reasons – see individual sample categories for details and reporting times.

Please mark **high risk samples** appropriately:

- Forms & sample bottles must be clearly marked with a red warning sticker.
- The sample must be sealed in a plastic bag. The accompanying form must not come into contact with the sample.

Request forms can be ordered from the Supplies Department at Ninewells, or a small number directly from the Genetics Specimen Reception (x33107)

UKNEQAS performance: CPA Accredited Scheme for Clinical Cytogenetics 2007:

The department participates fully in the UKNEQAS Scheme for Clinical Cytogenetics, and the most recent performance scores are given. The expected performance is “Satisfactory”.

CONSTITUTIONAL	PERFORMANCE	SCORE
Routine BLOOD	Satisfactory	100%
Urgent BLOOD	Satisfactory	100%
AMNIOTIC FLUID	Satisfactory	100%
CVS	Satisfactory	100%
HAEMATOLOGICAL		
MDS	Satisfactory	100%
CML	Satisfactory	100%
ALL	Satisfactory	100%
Total		100%

POSTNATAL CYTOGENETICS: (enquiries x36272)

Blood samples:

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

The [main referral categories](#) are:

- mental retardation or developmental delay
- infertility
- recurrent (3 or more) pregnancy losses
- problems of sexual development
- dysmorphism and congenital abnormality
- requests for specialist FISH studies (See page 9)

Reporting times:

- 28 days for routine samples
- 10 days for urgent referral
 - Neonates
 - in-patients under 1yr
 - parents of an ongoing pregnancy
 - specific clinical request.

[For urgent samples from the Neonatal Unit \(NNU\)](#) in Ninewells, a member of Genetics staff will be pleased to collect samples, if contacted on x33107/x36272

[For molecular genetic studies or DNA extraction, an EDTA sample is required \(see Molecular Genetics User Manual – consult the department if in doubt\)](#)

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 specimen bag
- With a completed Human Genetics form in the outer pocket (see General Notes)
- Samples from Ninewells may be delivered by the porter system
- Samples from PRI are sent via the Pathology transport system
- Samples from general practitioners are collected by van across Tayside

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 stored at 4°C.
- Samples delayed in transit may yield substandard results and require repeat sampling
- Samples of 1-2ml are acceptable from neonates, as are cord blood samples.
- For samples requiring specialist testing (eg: Fanconi anaemia) please contact the department before sampling

Limitations: Conventional cytogenetics will not detect chromosome abnormalities beyond the resolution of the light microscope. Molecular cytogenetics (FISH) can usually also be performed on these samples (see page 9) while array CGH studies will require an EDTA sample for DNA extraction (see page 11)

For solid tissue samples from pregnancy loss or termination see “Prenatal Cytogenetics” below.

PRENATAL CYTOGENETICS: (enquiries x36735)

Prenatal detection of chromosome abnormalities by fetal karyotyping at either amniocentesis or chorionic villus sampling. Most samples are received from Obstetrics clinics at Ninewells and Perth Royal Infirmary.

Amniocentesis sampling:

An amniotic fluid sample (~15ml) is taken trans-abdominally under ultrasound guidance, routinely at ~16 weeks gestation. Cells are cultured and karyotyped.

The [main referral categories](#) are:

- Increased risk of Down syndrome identified by maternal serum screening
- Maternal anxiety on the grounds of maternal age
- Abnormal ultrasound picture:
 - NB: All heart defect referrals are FISH tested for 22q11 microdeletion
- Family history of chromosome abnormality

Please liaise with the department for prenatal testing for other conditions.

Reporting times:

- available in 12-14 days - see note on rapid QF-PCR aneuploidy screening below

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

Sample requirements:

- amniotic fluid sample split in clinic between two labelled sterile universal containers:
 - 2ml to one tube, and the remainder to the other.
- Also a 5ml maternal blood sample into EDTA tube (**RED** or **PURPLE** top)
- accompanied by a Human Genetics request form (see General Notes)
- promptly dispatched to the laboratory:
 - Ninewells – hand delivery by Ultrasound staff
 - PRI – TAXI delivery to Ninewells Main Reception
- Gestational age and any relevant obstetric details or scan findings should be noted.

Suboptimal samples: the following samples may be unsuitable for QF-PCR and will have to await the conventional karyotype result. They may also have slightly longer reporting times

- small volume
- significant blood staining
- significant maternal cell contamination

Rapid Aneuploidy Screening (QF-PCR):

Allows rapid detection of Down, Edward and Patau syndromes on uncultured amniocytes and is applied to all amniotic fluid samples. A preliminary result is generally available within 24-72 hours of the procedure. However, it will not detect other types of chromosome abnormality.

QF-PCR involves DNA extraction from amniotic fluid, which is tested with a panel of markers, to determine copy number of the chromosomes of interest. Occasionally a sample will be uninformative for a particular chromosome, or because of maternal cell contamination. Often we can resolve this using a second marker set, but this may not always be possible. In all samples, the conventional karyotype from cultured cells will continue to provide a back up.

Rapid diagnosis of **Turner syndrome:** Amniotic fluid samples will not routinely be tested for fetal sex by QF-PCR, and Turner syndrome will not be detected.

If you suspect a Turner fetus from the ultrasound picture, please note this clearly on the referral form. We will then use an appropriate test. In cases with no scan indication, the Turner karyotype (or any other sex chromosome aneuploidy) will not be detected until the conventional cultures are available.

Chorionic Villus Sampling (CVS): enquiries x36765

A small sample of placental villi taken transabdominally under ultrasound guidance, CVS can be performed earlier in pregnancy than amniocentesis. Procedures are undertaken at Ninewells Diagnostic Ultrasound Department.

The [main referral categories](#) are:

- scan abnormality
- family history of chromosome abnormality
- prenatal diagnosis of a molecular genetic disorder (eg: 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:

- A preliminary “direct” result is usually available the following working day, which should detect numerical and gross structural chromosomal anomaly.
- However, this result is slightly less reliable than amniocentesis due to confined placental mosaicism, and confirmation from “long term” cultures takes up to 14 days. Patients should be counselled accordingly.

Sample requirements:

- Chorionic villus samples are accepted by prior arrangement with the laboratory.
- Sterile CVS collection medium containing heparin is available from the department, and should be prewarmed to room temperature before use.
- CVS collection medium is for **IN VITRO** use only
- Samples should be fully labelled, and accompanied by a Human Genetics request form (see General Notes)
- Samples **MUST** be promptly despatched to the laboratory (routinely hand delivered by a member of Ultrasound staff).

Suboptimal samples:

- Samples must contain sufficient identifiable placental villi (>10-20mg).
- Smaller samples 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 disorders require larger sample sizes. If the sample is insufficient, the patient may be required to wait for cultured samples to be extracted.

Limitations:

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

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

For technical reasons high-risk samples (eg: Hepatitis B, HIV), may be more prone to complications of maternal cell contamination. In these cases it may be a better option to consider amniocentesis sampling.

Solid Tissue Samples: (including TOP & pregnancy loss)

The [main referral categories](#) are:

- investigating causes of recurrent pregnancy loss (3 or more)
- fetal abnormality detected at post mortem or prenatal ultrasound
 - In cases of recurrent loss or fetal abnormality parental blood analysis may also be appropriate – consult the postnatal guidelines above
 - NB: All heart defect referrals are FISH tested for 22q11 microdeletion
- follow-up confirmation of prenatal findings on post-termination tissue

Also:

- mosaicism studies of skin in patients with normal blood chromosomes, when diagnosis is problematic
- culturing cells for molecular genetic or biochemical investigations

Sample requirements:

- Sterile tissue transport medium available on request (x 33107/36272)
- All samples should be accompanied by a Human Genetics request form (see General Notes)
- If prompt dispatch to the laboratory is not possible, samples should be stored at 4°C.
- **Fetal samples**
 - A sample of fetal tissue (e.g.: skin, muscle)
 - together with a separate sample of placenta (1cm³) should be sent from pregnancy losses and TOP's.

Fetal skin samples should be “full depth”, not “skin peel” samples. Macerated samples are NOT suitable for cytogenetics, and these cases require a placental sample only. For earlier losses, a sample of products of conception may be sent.

- **Skin Biopsies:**

Other samples of solid tissue should also be sent in transport medium, with full details of investigations requested, and any relevant family history. (Skin samples should be “full depth”).

These samples will routinely have chromosome analysis performed, and cultured material will be sent for DNA extraction or other studies (e.g.: biochemical assay) as required.

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 with 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](#) are usually available within 28 days.

Limitations:

- Chromosome analysis of pregnancy loss may not detect subtle chromosome abnormalities
- Macerated samples will result in sample failure
- Formalin fixed samples will not yield a result
- For technical reasons high-risk samples (eg: Hepatitis B, HIV) may be more prone to complications of maternal cell contamination.

ONCOLOGY CYTOGENETICS: enquiries 36272

Chromosome analysis in cancer allows the detection of chromosomal and genetic changes acquired during the disease process. We provide a routine service for leukaemia and lymphoma, supplying information for diagnosis, prognosis and disease management.

Bone marrow is the preferred sample in analysis of leukaemia and related disorders (eg: MDS), while for lymphoma, a fresh lymph node biopsy is required. Diagnostic leukaemic marrows are considered urgent. Other samples are considered routine, unless by specific clinical indication (eg: impending relapse or transformation). Peripheral blood samples are acceptable in chronic conditions (eg: CLL) or when marrow aspiration is difficult. Samples are taken in clinic at Ninewells or PRI.

All samples should be accompanied by a Human Genetics request form with:

- patient details
- clinical information including suspected disorder
- stage of disease (i.e: diagnosis, monitoring, relapse, post-BMT, etc.)
- any treatment
- any previous genetic studies

Sample requirements:

- **Prompt (same day) dispatch to the laboratory is essential; delay may compromise results.** Ninewells samples are generally delivered by hand. PRI samples are transported by van either from Pathology or Haematology.
- Bone marrow and lymph node samples are taken into heparinised transport medium supplied by the department on request).
- Peripheral blood samples are taken into lithium-heparin (DARK GREEN or ORANGE)

Reports:

- Urgent bone marrows may have preliminary results within 3 days (eg: rapid FISH)
- Karyotypes for urgent bone marrows are available within 14 days
- Karyotypes for routine bone marrows are available within 21 days
- Lymph node results are available within 21 days

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 ($>1 \times 10^6$ /ml) may not yield successful cultures.
- When no diagnostic chromosome studies are available, the analysis of disease monitoring samples is limited in the ability to detect clinically significant changes.

The cytogenetics Oncology service is supported by a comprehensive FISH service for the detection of genetic rearrangements in interphase and metaphase cells. In particular, we provide a routine service for the detection of **HER-2 amplification in paraffin sections**. These protocols can be applied to other tumour sections. Consult the relevant sections (Pages 9 & 10)

MOLECULAR CYTOGENETICS (FISH): enquiries x36272/x36735

Molecular cytogenetics (known as **F**luorescence **I**n **S**itu **H**ybridisation) can increase the speed, sensitivity and specificity of conventional cytogenetics. It can often be performed on the sample supplied for conventional cytogenetics, although sometimes further samples may be required.

The technique takes advantage of a property of DNA that 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 department for more specific details, as this is a rapidly expanding field.

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:

- Williams
- Prader-Willi/Angelman
- Miller-Dieker
- Smith-Magenis
- 22q11 deletion
- Wolf-Hirschhorn
- Cri-du-chat

This list is not exhaustive and more disorders are currently being investigated.

Aneuploidy screening:

A rapid approach to detect changes in chromosome copy number in a variety of clinical settings, without having to culture the cells (eg:detection of trisomy 21 in cord blood from a neonate; the acquisition of an extra copy of chromosome 12 in chronic lymphocytic leukaemia; or the detection of ploidy changes in fixed paraffin embedded tissue sections).

Detection of Gene Rearrangements in Cancer:

Oncogenic “fusion genes” may be created by rearrangement of the genetic material. This is a recognised cause of many cancers and can be highly specific. By use of two-colour FISH probes to both gene partners in a fusion (usually a cancer gene and a promoter gene) the novel sequences may be identified by the close juxtaposition of signals. The haematological malignancies have been the most extensively studied to date. However, these approaches can also be applied to paraffin sections (see [HER2](#) below)

Sample requirements:

FISH studies can usually be carried out on the same samples referred for conventional cytogenetics (except for HER2 studies – see below). Consult the individual sections above and telephone the department for advice in individual cases

Reports: are usually available within the reporting times for conventional cytogenetics. If they are required urgently they can be reported rapidly by telephone at the clinician’s request.

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 as applicable. If you wish to discuss the suitability of a particular sample type for testing, please call the department.

Note: **Subtelomere screening:**

This test is no longer routinely undertaken by FISH. Samples for testing require an EDTA sample and referral to the Molecular Genetics section for testing in Glasgow.

HER-2 Amplification status on paraffin embedded sections: x36272/x36735

Detection of HER-2 amplification status in breast tumour is an indicator for Herceptin therapy, whilst non-amplified tumours derive no benefit from this treatment. We provide a service for the detection of HER-2 amplification on paraffin sections.

Sample requirements:

- 3-5 x 4µm sections on positively charged slides (with a white "+" on the end).
- Slides must have been baked overnight at 56C.
- It is important to not over formalin treat slides (24-48hrs maximum).

Slides should be clearly marked with the identifying pathology number and patient name. The accompanying paperwork must provide:

- Patient prename and surname,
- DOB & CHI if known
- Pathology number
- ICC score if known
- Referring clinician
- Hospital address
- Telephone number or bleep.

Slides from Ninewells are delivered by hand. Slides from other hospitals (eg: Raigmore) should be posted/couriered in appropriate slide holders and padded envelopes to avoid breakages and be accompanied by completed request forms. Notification of Her-2 cases or other tissues coming from outwith Ninewells hospital should be emailed to the contact person in the FISH department. On receipt of sections the sender will be emailed to inform that they have arrived safely. Urgent cases should be clearly marked.

Reports:

Her-2 test results are normally available within 10 working days. These are posted first class although arrangements can be made to send to a secure FAX if required urgently.

This protocol can be applied to other tumour sections e.g IGH/BCL2 status for lymphoma sections where fresh material is not available. Please consult the department for details of probes available.

Limitations:

Samples are likely to be unsuccessful if the sections:

- Have had prolonged fixation (over 48 hours)
- Are not on positively charged slides
- Show extensive areas of necrotic or fatty tissue
- Show no areas of identifiable tumour tissue

Array Comparative Genomic Hybridisation: (enquiries x36735)

During 2007 the department introduced the new technique of array comparative genomic hybridisation (aCGH), also known as microarray or CHIP analysis. For constitutional cases tests would require referral via Consultant Clinical Geneticists as gatekeepers of the service. Diagnostic criteria are generally for children with moderate to severe retardation and associated significant malformation. Results may take up to three months, although are usually turned around more quickly. The following description of the test is included on all aCGH reports.

“Basis of aCGH test:

Array comparative genomic hybridisation is performed using a BlueGnome CytoChip array. Test DNA is referenced against same sex control DNA in dye-swap hybridisations and data analysed with BlueFuse software. Copy number changes are sized according to DECIPHER convention.

This is a new diagnostic test for constitutional studies, which is still under development, and there are currently no nationally accepted best practice guidelines. However, using the analysis protocol described above, we are confident of the quality of this result. The array has a backbone clone set with higher coverage across known disease related regions, and subtelomeric regions. The resolution of the arrays is increased periodically as new versions are brought out, and this is documented in all clinical reports. Please note aCGH will not detect balanced rearrangements and is limited in detection of mosaicism”

Other types of array (eg: cancer, microdeletions) are under development, and we will keep you informed of progress in these areas.

Sample requirements:

- Referral by a Consultant Clinical Geneticist.
- 2-5ml whole blood in EDTA (RED or PURPLE top)
- If the test is on a new specimen, rather than an archival sample, it would be advantageous to send 2-5ml lithium-heparin at the same time.
- We would expect these referrals to have previous normal karyotype studies

Reporting:

A sample report is presented above. Most are expected within three months, although straightforward samples are likely to be reported more quickly.

Limitations:

- The success of the technique is largely determined by the quality of the DNA obtained with respect to:
 - DNA volume
 - DNA concentration
 - DNA integrity
 - DNA purity
 This is subject to stringent Quality Control criteria. Specimens not fulfilling these criteria are likely to require further sampling.
- It will often be necessary to request parental blood EDTA and lithium-heparin samples to interpret an aCGH result. If either parent is unavailable, it may not be possible to reach a definitive conclusion as to whether a change is causal or a clinically insignificant polymorphism
- Array CGH will not detect balanced rearrangements and is limited in the detection of mosaicism. For these reasons there may be cases where a different strategy is more appropriate. Please contact the department if you wish to discuss these tests.

RESEARCH AND DEVELOPMENT:

NHS Cytogenetics has been well supported by NHS Tayside Group Directorate of Surgery and Oncology and translational cancer research and cutting edge cancer diagnostic services are recognised as a particular Tayside strength. To this end Cytogenetics has maintained a longstanding interest in Molecular Cytogenetic R&D with a view to seeing these advances speedily incorporated into improved service provision.

The Department is focussed on 2 complementary areas of genetic investigation: FISH on paraffin embedded tissue (PET) and Array-CGH. The latter has been a natural extension of conventional metaphase CGH for which the department had particular expertise. As with all successful clinical cancer research this has involved a multidisciplinary effort with other Medical School and University of Dundee colleagues. Currently, we have 3 clinical areas of interest, B-cell lymphoma, breast cancer and colo-rectal cancer. Each of these is underpinned by Grant funded research and a dedicated PhD or Post-Doctoral Scientist. Students are co-supervised by senior NHS/University Staff. We have been able to publish on a regular basis in good quality journals and present at appropriate National and International conferences. Finally, because all of our R&D effort is closely aligned to clinical need this can quickly inform new or improved services to patients