

S.W.Thames Regional Genetic Service
At St.George' s Healthcare NHS Trust

**Information for Users of the
Cytogenetic Service**

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Introduction

The Cytogenetic Unit is situated in Jenner Wing of St. George' s Hospital and is one of the Four London based Laboratories providing a comprehensive range of cytogenetic tests to patients in London, Bedfordshire, Essex, Hertfordshire, Kent, Surrey, Middlesex and Sussex.

The Postal Address is:

Regional Cytogenetic Unit
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Following the retirement of the previous head of laboratory. The duties of this position are currently being managed jointly by:

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The Cytogenetic Unit provides a comprehensive service for the investigation of constitutional chromosome abnormalities. Some solid tumour work is undertaken, and fibroblasts can be cultured for biochemical and/or DNA studies

The Cytogenetic Unit is open Monday to Friday between the hours of 9.00 am and 5.00 pm. There will not normally be anyone in the department outside these hours or over Bank Holiday periods. Advice may be sought about any urgent samples arriving outside these hours by contacting the laboratory during its usual opening hours.

1 Request Forms.

Requests for Cytogenetic tests should be accompanied by a Cytogenetic request form whenever possible. (The laboratory request form is available for download from the website) All request forms should be completely and clearly filled out including:

- Correct patient identification including full name, date of birth and address.
- Sample type with date and time of collection.
- NHS number, and where applicable local patient identifier (e.g. hospital number)

- Name and address of referring doctor.
- Patient's GP and the PracticeCode
- Clear indication as to why a test has been requested.
- If a full karyotype/Array CGH and/or FISH test is required. (Please specify what specific FISH test if any).
- Consent for testing and possible storage of material
- Status: NHS or Private

Please note that for an Array CGH test there is an extra form, available on the website that must be completed.

Failure to fill in the request form clearly and correctly may result in inappropriate tests and delays in reporting. Serious problems in patient information may result in the sample not being processed.

2 Sample requirements:

NO SAMPLE FOR CHROMOSOME ANALYSIS SHOULD BE FROZEN OR PLACED IN ANY PRESERVATIVES OR FIXATIVE

2.1 Venous Blood:

For Chromosome analysis (Karyotyping) 4ml is required (1ml will suffice for small babies) unclotted in a Lithium Heparin container (green top vacutainer or orange screw cap)

For **Array CGH** 4-8ml in EDTA is required.

2.2 Fetal Blood:

For **Chromosome analysis (Karyotyping)** at least 1ml is required, unclotted, in a Lithium heparin container

For **Array CGH** at least 1ml is required in an EDTA container.

Please note that Microtainer brand 0.6ml Lithium heparin bottles do not give good results so please avoid using these where possible.

2.3 Amniotic Fluid:

10 –20 ml is required in a sterile universal container.

2.4 CVS Biopsies:

10-15mg is required (more if the sample needs to be split for other tests) in CVS transport medium, (samples less than 5mg may only be sent for QFPCR and no cultures set up, clinicians will be informed.).

Transport medium will be supplied by the laboratory on request.

2.5 Solid Tissues and Tumours:

Samples should be sent in a sterile container in transport medium available on request from the laboratory. If medium is not available **sterile isotonic saline** may be used. Dry samples, if received the same day, are also acceptable.

2.6 Any other samples:

Please phone the Unit to discuss requirements.

All samples should be sent to the Cytogenetic Unit as soon as possible after collection.

Prenatal samples should arrive in the department by 4.00. p.m. on the day of sampling whenever possible. First class post is acceptable but will add to the reporting time. Posting over a weekend should be avoided if at all possible.

Peripheral blood samples can usually be sent by first class post. Avoid posting over a weekend whenever possible as samples may deteriorate if delays occur in transit.

All samples must be clearly labelled. When sending samples, please make sure that the containers are tightly capped and well packed with a separate pocket for the request form. All samples must be packaged to comply with current legislation. The address for specimen reception is as printed above.

If you have any queries concerning samples for chromosome analysis please contact the laboratory on 020 8725 5332.

3 Acceptance criteria for samples.

Samples are subject to the acceptance criteria adhered to by this laboratory, and the sample will not be processed if it does not meet these criteria. If a clinician feels that there is a good clinical reason to send a sample which does not meet our acceptance criteria they should discuss this with the laboratory staff prior to sending the sample so that agreement can be reached.

Private Patients

Patients undergoing investigations as part of a private consultation fall outside the scope of the specification and are regarded as private referrals and test costs will be invoiced to the requesting clinician/patient/other as appropriate. If the referral falls outside the usual laboratory criteria for NHS samples please phone the laboratory to discuss requirements before sending the sample for processing.

3.1 Indications for Prenatal Karyotyping

Sample types: amniotic fluid, chorionic villous, fetal blood.

3.1.1 Referrals that will have routine rapid screening for common trisomies using QFPCR only.

- High risk of aneuploidy from the result of a screening programme as defined by the UK National Steering Committee (NSC) current standards. **These samples will have QFPCR testing for trisomy 13, 18, and 21 only. Material will be kept short term to check any abnormal or equivocal QFPCR results with a full karyotype as necessary.**
- Pregnancies at a prior risk of a single gene disorder. **These samples will not be routinely karyotyped, but will be screened for trisomies 13, 18, and 21 using**

QFPCR in addition to testing for the single gene disorder in question. Cultures will be set up to provide further material for testing as required and used to check any abnormal or equivocal QFPCR results with a full karyotype as necessary.

- **Samples obtained while undergoing other invasive procedures, e.g. transfusion for RhD.**

Notes

- *Risks based on Maternal Age alone should not be used.*
- *QFPCR testing will include screening for sex chromosome aneuploidy where:*
 - *The Nuchal Translucency is 4mm or greater*
 - *There is a risk of an X linked disorder*
 - *There is ultrasound detection of abnormalities such as cystic hygroma or cardiac abnormalities indicative of Turner syndrome*
 - *Ultrasound evidence of ambiguous genitalia*
 - *Knowledge of the sex may facilitate counselling and/or allow for targeted investigations*

3.1.2 Referrals that will have full karyotyping and QFPCR.

- Ultrasound detection of any major structural abnormality including nuchal translucency (NT) >3mm before 14 weeks gestation or a nuchal fold measuring 6mm or greater between 14 and 20 weeks gestation.
- Ultrasound detection of two or more minor markers of aneuploidy. Consistent with NSC guidelines, Karyotyping should not be offered on a single marker in women who have a low risk following aneuploidy screening.
- History of chromosome abnormality indicative of increased risk for future pregnancies. Chromosome abnormality may be present in:
 - ❖ the woman or her partner
 - ❖ a previous pregnancy (excluding non-viable aneuploidy)
 - ❖ If there is a family history, karyotyping of the woman or her partner should be undertaken first in order to establish whether prenatal diagnosis is indicated.
- Non-routine cases not fulfilling the above criteria after discussion and agreement between the referring clinician and a senior staff member from Cytogenetic Unit.

NOTES

Maternal anxiety is not an indication for prenatal karyotyping unless considered by the referring clinician to be important for the management of the pregnancy.

All prenatal samples of amniotic fluid or CVS accepted for full karyotyping are routinely sent for QFPCR for rapid diagnosis of common trisomies 21, 13 and 18.

QFPCR testing is sent to another currently CPA accredited laboratory at Guy's hospital who issue their results directly to the referrer.

Refs:

1) Ultrasound Screening Supplement - to Ultrasound Screening for Fetal Abnormalities RCOG 2000. (<http://www.rcog.org.uk/womens-health/clinical-guidance/ultrasound-screening>).

2) Normal variant screening in pregnancy NHS Screening Programmes – Fetal Anomaly – Programme Statement on: ‘ Normal Variant (‘ previously known as soft markers’).

This statement contains a note that the information therein supersedes ‘ *Ultrasound for Screening for Aneuploidy: Guidance for the professional*’ (RCOG 2000).

3) QF-PCR Health Professional Information Leaflet ‘ *Information for Health Care Professionals – Guidelines for QF-PCR testing alone for women at increased risk for Down’s syndrome*’ 2007.

UK National Screening Committee current guidance for Testing for Down’s Syndrome in pregnancy

3.2 Indications for paediatric/adolescent karyotyping

Sample type: Mostly Peripheral blood but may be Solid Tissue (skin biopsies).

- Unexplained learning difficulties/developmental delay. (Array CGH is now considered to be the frontline test for these referrals though Karyotyping may been done **instead** at the clinicians request. Please see below).
- Dysmorphism/multiple congenital abnormalities suggestive of a chromosome abnormality (Array CGH may be more appropriate for some of these cases as a frontline test. Please phone for advice if required.)
- Ambiguous genitalia/indeterminate gender.
- Delayed puberty, or inappropriate secondary sexual development.
- Family history of a chromosome abnormality
- Short stature, amenorrhoea in females.
- Microdeletion/duplication syndromes (includes FISH testing if probes are available and if diagnosis by molecular genetic methods is not locally available).
- Cases not fitting within these categories after discussion and agreement between the referring clinician and senior laboratory staff.
- Confirmation of a diagnosis made prenatally

3.3 Indications for adult karyotyping

Sample type: Mostly Peripheral Blood but may be Solid Tissue (skin biopsies).

- Any of the paediatric/adolescent referral categories.
- Oligozoospermia or azoospermia in males.
- Premature ovarian failure.
- Parental karyotyping after three or more unexplained miscarriages where fetal karyotyping has not been done, was not possible, or has been unsuccessful.
- Parental karyotyping after pregnancy loss of an unkaryotyped fetus with multiple congenital abnormalities or severe IUGR or unexplained stillbirth/neonatal death.

- Family history of a known chromosome abnormality other than simple aneuploidy due to non-disjunction.
- Suspected family history of chromosome abnormality where the karyotype of the affected individual is not known.
- Sperm and egg donors for NHS funded patients.
- Couples undergoing assisted conception funded by the NHS.

NOTE: *Parental karyotyping for a family history of a chromosome abnormality where karyotyping has shown aneuploidy from non disjunction (typical primary trisomy) is not an appropriate referral request.*

3.4 Indications for post mortem perinatal, fetal or placental karyotyping

Sample types: Solid tissues such as: products of conception, fetal/neonatal tissue, placental tissue, or fetal/neonatal blood.

- Fetuses/stillbirths/neonatal deaths with congenital abnormality suggestive of a chromosomal anomaly or with neural tube defect or with IUGR.
- Follow up of prenatal ultrasound findings suggestive of a chromosome anomaly.
- Follow up confirmation of prenatal cytogenetic findings on post termination tissue.
- Unexplained stillbirth or neonatal death (≥ 24 weeks).
- Unexplained IUD/spontaneous abortion (≥ 16 weeks).
- Spontaneous abortion where the couple has a known chromosome rearrangement.
- Spontaneous abortion less than 16 weeks where the couple were undergoing assisted conception funded by the NHS.
- In the case of unexplained early miscarriage (<16 weeks) samples of fetal tissue should **not usually** be sent except on the third or more occurrence of unexplained early miscarriage.
- Cases of perinatal death under investigation by the coroner.

3.5 Supplementary and/or specialised tests

Cytogenetic testing is often limited to a full analysis of the patient's G-banded metaphase chromosomes; however, in some circumstances more specialised techniques are required. The following list indicates the supplementary tests that will routinely be applied when necessary for full interpretation of results.

- Extra specialised conventional staining techniques (e.g. C-banding)
- Chromosome breakage analysis for diagnosis of chromosome instability disorders (These will be sent to another accredited laboratory for testing).
- Routine application of commercially available FISH probes including whole chromosome painting probes, centromeric probes, sub-telomeric probes and locus specific probes (for microdeletion syndromes).
- Rapid testing by interphase FISH for urgent neonatal referrals for sex ascertainment and to test for trisomy 13, 18 and 21.
- Diagnosis of cryptic chromosome anomalies by sub-telomere screening.
- Mosaicism screening of skin samples from patients with a normal blood karyotype

3.6 Solid Tumours

Some solid tumour work is done routinely on certain paediatric tumours. FISH for MYCN status, deletion of 1p36, and 17q gain can be carried out on neuroblastomas. For all tumours outside St. George's, the availability of testing should be discussed before sending samples.

3.7 Array CGH (NEW SERVICE)

We are now offering Array CGH as a **first line test** for paediatric and adult samples with the following referral reasons.

- Developmental delay, learning difficulties, speech/language delay (with or without autism).
- Dysmorphic facial features
- Multiple congenital abnormalities with or without pre/postnatal growth abnormalities.
- Apparently balanced chromosome rearrangements with an abnormal phenotype.

This is a **new service** and letters explaining this have been sent out to the majority of repeat users of our service.

Blood samples are requested in EDTA

Family follow up will be requested to clarify the significance of any finding where the association with the phenotype is not initially clear. Parental samples and any further patient sample required will be requested on the preliminary report and follow up will be done using a variety of techniques such as Fluorescent in situ hybridization (FISH), MLPA or parental arrays.

Please note that if Array CGH **and** Karyotyping are requested, the karyotyping will not normally be done as imbalance in patient genome will be detected more accurately by array CGH. Karyotyping of the patient may be done at the laboratory's discretion to give additional information when this would be useful for interpretation of the array result.

Array CGH will also be offered for certain prenatal samples when there is agreement for this with one of the Genetic consultants in the department. The sample requirements will be discussed for individual cases with the referring clinician.

Please note that for an Array CGH test there is an extra form, available on the website that must be completed.

Please phone and ask about any tests, which may have recently become available.

4 Storage of clinical material following analyses.

Storage will meet the minimum guidance in 'The retention and storage of pathological records and archives (4th Edition, 2009). Guidance from The Royal College of Pathologists and the Institute of Biomedical Science'.

Storage of tissue will be in accordance with the requirements of The Human Tissue Act (2004).

There will routinely be:

- Storage of fixed cell suspensions for future testing.
- Storage of slides/digitised images.

And on request

- Storage of viable fibroblast cultures at -80°C .

Individual requests for non routine storage will be considered depending on facilities and material available.

5 Responsibility for obtaining consent.

- It is the responsibility of the clinician who requests the test to ensure that the patient has given informed consent for the test to be carried out and for material to be stored where necessary.

6 Responsibility for transport of samples

- It is the responsibility of the referring centre to ensure that samples arrive at the laboratory in suitable condition i.e. promptly, in the correct container, packaged and transported according to current legislation, labelled with the patient's name and a second identifier e.g. date of birth or hospital/NHS number and accompanied by a completed request form.

7 Unsuitable samples

- The laboratory has clear procedures for dealing with unsuitable samples which include liaison with the referring clinician and issuing of a written report.
- Incorrect labelling of sample (i.e. discrepancy between name on sample and name on referral form or no name on sample or no name on referral form). Following discussion with the referring clinician if the labelling discrepancy cannot be resolved, the sample may not be processed.
- Sub-standard samples. Culturing may be attempted if repeat sampling would not be appropriate or possible as in the case of a prenatal diagnosis sample or death of the patient e.g.
 - Sample in wrong tube.
 - Sample clotted.
 - Sample delayed in transit for more than 5 days.
 - Non-viable sample (lysed, frozen, fixed).
 - Broken sample tube.
- High-risk samples requiring containment level 3 facilities e.g. known cases of CJD/vCJD, TB cannot be processed.
- Samples with inappropriate referral reasons may not be processed.

8 Disposal of samples

Samples will be disposed of according to national guidelines.

9 Reports

- Final reports will be issued when chromosome or array CGH analysis is complete. Reports will include a karyotype or array result using current ISCN where appropriate, and a clinically relevant interpretative comment and reference to the inclusion of relevant literature where appropriate.
- All prenatal and neonatal reports will be promptly communicated to the referring centre by telephone and/or fax.

10 Turnaround Time

The laboratory will aim to provide reports within the current reporting guidelines indicated by ACC Best practice guidelines in Clinical Cytogenetics, or agreed with the Genetics Consortium. These are as follows:

CVS and Amniotic fluid samples	14 days
Urgent bloods	10 days
Non urgent postnatal bloods	28 days
Solid tissues	28 days
Solid tumours	no guidelines available but we attempt to report within 28 days.
Array CGH	8 weeks
Array CGH follow up where necessary	8 weeks

As reporting times do vary depending on work pressures the current reporting times are displayed on our website.

11 Audit of Outcome

- Pan-London audits of referral patterns will be undertaken in liaison with commissioners.
- Data will be provided for data monitoring and/or chromosome anomaly registers as required.

12 Quality Assurance

The laboratory participates in an external quality assessment scheme relevant to the test repertoires (UK NEQAS in Clinical Cytogenetics).

The laboratory complies with Best Practise Guidelines issued by various professional bodies.

This Laboratory is CPA accredited.