REGIONAL CYTOGENETICS LABORATORY FOR WALES

USER MANUAL
CONTENTS

INTRODUCTION ......................................................................................................................... 4

SENDING SAMPLES TO THE LABORATORY ........................................................................... 6

SAMPLE REQUIREMENTS .......................................................................................................... 6

POSTNATAL BLOOD .................................................................................................................. 6

PRENATAL SAMPLES .................................................................................................................. 6

- Amniotic fluid .......................................................................................................................... 6
- Chorionic villus sample (CVS) .................................................................................................. 6
- Foetal blood .............................................................................................................................. 6

SOLID TISSUES / FOETAL MATERIAL & POCs.................................................................... 6

- Spontaneous abortions / products of conception (POCs) ...................................................... 6
- Couples with recurrent pregnancy loss .................................................................................... 6
- Abortuses and stillbirths ........................................................................................................... 6
- Terminations of pregnancy ...................................................................................................... 7
- Samples required from foetuses and stillbirths ....................................................................... 7
- Tissue biopsies .......................................................................................................................... 7
- Solid tissue sample / container / media requirements .............................................................. 7
- Solid tissue sample dispatch ................................................................................................... 8
- Disposal of tissue ..................................................................................................................... 8

BONE MARROW / LEUKAEMIC BLOOD SAMPLES ................................................................. 8

INSTRUCTIONS FOR SENDING SAMPLES SAFELY ............................................................... 9

DIAGNOSTIC SAMPLES ............................................................................................................ 9

PACKAGING INSTRUCTION 650 FOR DIAGNOSTIC SAMPLES .............................................. 9

1. Primary receptacle .................................................................................................................. 9
2. Secondary packaging .............................................................................................................. 9
3. Outer packaging ..................................................................................................................... 9

ADDITIONAL IMPORTANT INFORMATION REGARDING SAMPLES SENT TO CARDIFF ....... 10

FORWARDING SAMPLES TO OTHER LABORATORIES .......................................................... 11

TEST REPERTOIRE OF CYTOGENETICS LABORATORY ......................................................... 12

CYTOGENETICS ......................................................................................................................... 12

- Blood sample indications ....................................................................................................... 12
- Bone marrow and leukaemic blood sample indications ........................................................ 12
- Amniotic fluid, CVS and foetal blood sample indications ...................................................... 12
- Fibroblast culture (from skin and other solid tissues) indications ........................................... 13

MOLECULAR CYTOGENETICS ................................................................................................. 13

- Fluorescence In Situ Hybridisation (FISH) ............................................................................. 13
- Identification of visible imbalances ......................................................................................... 13
- Microdeletion detection ........................................................................................................... 13
- Sub-telomere screening .......................................................................................................... 14
- Malignancy diagnostics .......................................................................................................... 14

QF-PCR Rapid aneuploidy testing ............................................................................................ 14

- Amniotic Fluid Samples QF-PCR ......................................................................................... 14
- Chorionic Villus Samples QF-PCR ....................................................................................... 15
- Solid Tissues QF-PCR ............................................................................................................ 16
- Blood Samples QF-PCR ......................................................................................................... 16

MLPA ......................................................................................................................................... 16

- Microarrays ............................................................................................................................. 16

REPORTING TIMES, SUCCESS RATES AND OTHER REPORTING ISSUES ....................... 18

SAMPLE REPORTING TIMES .................................................................................................. 18

CULTURE SUCCESS RATES .................................................................................................. 19

AUDIT AND QUALITY ASSURANCE .................................................................................... 19
Prenatal outcome.......................................................................................................................... 19
Haematological / malignancy follow-up..................................................................................... 19
POSSIBLE REPORTING COMPLICATIONS IN CYTOGENETICS............................................. 20
PRENATAL SAMPLES.................................................................................................................. 20
  Amniotic fluid and CVS QF-PCR samples.............................................................................. 20
  Amniotic fluid culture samples............................................................................................... 20
  Chorionic villus samples (CVS)............................................................................................. 21
POSTNATAL SAMPLES............................................................................................................... 21
  Blood samples......................................................................................................................... 21
  Solid tissue samples............................................................................................................... 21
USEFUL CONTACT NUMBERS................................................................................................ 23
INTRODUCTION

The Regional Cytogenetics Laboratory for Wales provides a comprehensive cytogenetics service for the Welsh population of almost 3 million people. It is housed in purpose-built accommodation in the Institute of Medical Genetics, University Hospital of Wales, Cardiff. Cytogenetic testing involves analysing human chromosomes by karyotyping and molecular cytogenetic techniques, including FISH, QF-PCR, MLPA and array CGH, at the microscopic and sub-microscopic levels. These valuable tests allow detection of chromosome abnormalities which cause serious malformation syndromes, developmental delay and other disorders. This service includes cytogenetic investigation of:-

- **Prenatal** amniotic fluid, cord blood and chorionic villus samples from women at risk of having children with chromosome abnormalities, such as Down syndrome, by karyotype analysis, coupled with rapid aneuploidy testing by QF-PCR and interphase FISH analysis;

- **Postnatal** blood and skin samples from children and adults with congenital abnormalities, learning difficulties, infertility, etc.;

- **Post-mortem** samples, from terminations of pregnancy and products of conception; and

- **Haemato-oncology** samples, mainly from patients with leukaemia and allied disorders.

To achieve this, the Laboratory has an establishment of 32 scientific, technical, and clerical staff. Clinical advice and interpretation of cytogenetic abnormalities is readily available to referring clinicians from Consultant Grade and Principal Grade Cytogeneticists to assist in the clinical management of affected cases and counselling of families. Clinicians should ensure that appropriate consent is obtained from the
patient or parent/guardian for testing and disposal of any spare tissue. The Laboratory is accredited by Clinical Pathology Accreditation Ltd. (CPA) and participates in relevant modules of the United Kingdom National External Quality Assessment Schemes (UKNEQAS) in Clinical Cytogenetics.
SENDING SAMPLES TO THE LABORATORY

SAMPLE REQUIREMENTS:

POSTNATAL BLOOD

Conventional cytogenetic analysis
– Blood in lithium heparin (5 ml*). Peripheral blood is the tissue of choice for determining a constitutional karyotype.

Molecular cytogenetic FISH analysis
– Blood in lithium heparin (5 ml*)

Molecular cytogenetic array CGH, MLPA and QF-PCR analysis -
Blood in EDTA (5 ml*)

* Infants: minimum of 0.5 ml

PRENATAL SAMPLES

Amniotic fluid
(15 – 20 ml)

Chorionic villus sample (CVS)
– by arrangement (a maternal blood sample (5 ml) in EDTA is also required in each case to determine the maternal genotype)

Foetal blood
– by arrangement

SOLID TISSUES / FOETAL MATERIAL & POCS

Spontaneous abortions / products of conception (POCs)
Cytogenetic investigations are not normally carried out on early abortuses and products of conception, in the absence of foetal abnormalities, other than in exceptional circumstances, e.g., where a parent is a known translocation carrier.

Couples with recurrent pregnancy loss
For couples who have experienced more than two spontaneous abortions, regardless of whether they have had any successful pregnancies, parental blood karyotyping is indicated. Samples required:
• blood (5 ml) from both partners

Abortuses and stillbirths
We accept samples where the infant or foetus has malformations (other than an isolated neural tube defect), dysmorphism or IUGR, and where a chromosome abnormality has been
detected prenatally. Full clinical details should be given.

**Terminations of pregnancy**
We accept samples from terminations of pregnancy carried out where a chromosome abnormality has been detected prenatally and / or where foetal abnormalities have been detected on ultrasound scan. Full clinical details should be given. Consent should be obtained for testing. Confirmation of anomalies detected prenatally may be obtained by use of QF-PCR and /or interphase FISH probes specific for the chromosome involved.

**Samples required from foetuses and stillbirths**
If the infant / foetus appears fresh and there are no signs of maceration, please send:
- cord blood or peripheral blood in lithium heparin and
- a sample of skin or other solid tissue

If the foetus appears slightly macerated, please send:
- a sample of foetal membranes (amnion) and / or placenta

Please do not send samples from infants or foetuses that show obvious signs of maceration.

**Tissue biopsies**
We accept samples from children and adults for cell culture where there is suspicion of a chromosome abnormality (usually in mosaic form), a genetic condition or a metabolic disorder. Where indicated, cultures of cells are forwarded to an appropriate Genetics or Biochemistry Department, an address, telephone number and contact name for which must be provided on the request form. A billing address and cost code must be provided for all such biochemical investigations before the dispatch of cultures. Please indicate on the request form if you wish the fibroblast cultures to be frozen down for storage for future testing.

**Sample required:**
- a suitable small biopsy of skin or other solid tissue (see below)

**Solid tissue sample / container / media requirements**
Samples must be placed in transport medium in a sterile Universal container, available from the Cytogenetics Laboratory, and sent to the Laboratory, without delay, by hospital transport or by taxi. (In the absence of specific transport medium, please use sterile viral transport medium or, failing that, sterile normal saline.)

Cytogenetic (karyotype) investigations can only be carried out on live healthy tissues. Therefore:

- the sample must not be frozen
- the sample must not be placed into formalin or any other fixative solution or any container that was used to hold fixative
- skin samples must include the dermis and associated underlying tissue
- ensure sterile / aseptic procedures are followed when
taking samples to avoid infection of the cultures

**Solid tissue sample dispatch**

Samples should be sent to the Laboratory without delay. Outside the working day, on weekends or on Bank Holidays the sample may be kept in a refrigerator at +4°C in transport medium, and sent to the Laboratory as soon as possible.

We do not generally accept samples that are more than 5 days old.

**Disposal of tissue**

Any spare tissue and spare cultures will be disposed of appropriately. Remnants of POCs will be disposed of sensitively following a protocol approved by the Trust, unless the patient requests that any spare material is returned. Please obtain consent from the patient for disposal of spare tissue and remnants of POCs. Whole foetuses should be sent to the Foetal Pathology Unit (FPU) at the Institute of Medical Genetics, not to the Cytogenetics Laboratory. These should be accompanied by completed consent and request forms, which are available from the FPU. Samples for tissue culture should be taken before sending the whole foetus to the FPU to ensure the best chance of successful culture.

Please contact the following personnel at the Cytogenetics Laboratory if you have any enquiries regarding sending solid tissue samples:
Merryl Curtis, telephone no. 029 2074 4024, or Lindsay Williams, telephone no. 029 2074 4071.

**Bone marrow / leukaemic blood samples**

Bone marrow samples should be taken into Universal containers containing bone marrow transport medium supplied by the Laboratory. These are issued to users once every two weeks with an expiry date of 3 weeks from the time of dispatch.

The transport medium should be stored refrigerated but not frozen. Please do not use medium that has passed its expiry date, although there is a fairly generous safety margin beyond the limit specified. If you wish to be placed on the mailing list for bone marrow transport medium or you wish to increase or decrease the number of containers you receive, please contact the Laboratory on the telephone number given below.

In an emergency, samples may be placed into a lithium heparin tube and carefully mixed with the anticoagulant.

Bone marrow is the tissue of choice for investigating most leukaemias and allied haematological disorders, although peripheral blood may yield results if blasts are present.

However, for chronic lymphocytic leukaemia peripheral blood is required in preference to bone marrow.

Peripheral blood (5 ml) should be sent in a lithium heparin tube.

Please indicate as precisely as possible the disease type and for lymphoproliferative disorders, whether they are likely to be derived from T or B-cells, as this information will help determine the culture regimens employed, including the choice of mitogen.
All bone marrow and leukaemic blood samples should ideally be sent without delay by taxi or hospital transport to the Laboratory.

INSTRUCTIONS FOR SENDING SAMPLES SAFELY

DIAGNOSTIC SAMPLES

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 (see below). Such packaging may be specially purchased for this purpose or constructed from suitable components.

PACKAGING INSTRUCTION 650 FOR DIAGNOSTIC SAMPLES

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 50 ml or 50 g, individually wrapped with enough absorbent material to absorb all fluid in the event of leakage or breakage.

2. **Secondary packaging**, durable, and leakproof, to enclose and protect primary receptacle(s). Multiple individually wrapped primary receptacles may be placed in one secondary packaging. Sufficient additional 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** must be followed:-

- Place the sample tube/Universal container in the leak-proof plastic bag with integral sealing strip attached to the Laboratory Request Form
- Seal the plastic bag
- Wrap enough absorbent material, e.g., cotton wool or paper tissues, around the plastic bag and tube/Universal container to absorb all fluid in the case of breakage
- Place in suitable secondary packaging, e.g., robust, leak-proof, screw-cap plastic container with a waterproof gasket/seal
- Place any additional paperwork within the container, but not
within the plastic bag containing the specimen tube/Universal container

- Identify high risk of infection samples and Request Forms with ‘Danger of Infection’ labels

- Close the screw-cap of the secondary packaging firmly

- Place the secondary packaging in suitable outer packaging, e.g., large padded bag, close-fitting rigid cardboard box, or cardboard box with polystyrene foam insert

- The name and address of the Laboratory should be clearly shown on the outside of the outer packaging

All samples should be forwarded to:

Cytogenetics Laboratory Service  
Laboratory Genetics Service for Wales  
Institute of Medical Genetics  
University Hospital of Wales  
Heath Park  
Cardiff, CF14 4XW

- The outer packaging should be clearly labelled:

**URGENT DIAGNOSTIC SPECIMEN**

**FRAGILE HANDLE WITH CARE**

- The outer packaging should be clearly marked:

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**ADDITIONAL IMPORTANT INFORMATION REGARDING SAMPLES SENT TO CARDIFF**

*Please complete a Genetic Diagnostic Laboratory Request Form ensuring that all the information requested on the form is provided (if no form is available, please call 029 2074 4023 for a supply: in an emergency a request form can be printed from the Medical Genetics web site on the Cardiff and Vale UHB Intranet or from*
the Cytogenetics Department pages of the Institute of Medical Genetics website, http://www.wales.nhs.uk/awmgs, in particular, provide full patient details including the NHS Number (please apply patient addressograph label if available), sufficient information about the referring consultant to allow the report to be returned without delay, specify clearly which tests are being requested, and provide all relevant clinical details.

For prenatal samples, please provide the name of the operator, i.e., the person taking the sample, and the gestational age.

All cytogenetic investigations require living cells. It is therefore important that we receive the sample as soon as possible after it is taken. First class post is usually satisfactory for blood samples. Special arrangements, e.g., by courier, Royal Mail Special Delivery, ambulance service, or taxi, should be made for prenatal samples, urgent blood samples and all other types of samples.

Experience has shown that chromosome results may not be obtainable from samples arriving at the Laboratory more than 4 days after collection from the patient. By this stage, cells, including peripheral blood lymphocytes, do not grow adequately in culture. Samples greater than 5 days old on receipt will not normally be processed.

Samples for cytogenetic investigations should not be frozen or exposed to excessive heat. If samples need to be stored, e.g., overnight, please keep them in a refrigerator (+4°C) or, or failing this, in a cool place.

Please note that mail is not delivered to the Laboratory on Bank Holidays. Please ensure that samples are taken and dispatched to arrive well before a Bank Holiday. Do not send samples by first or second class post in the two weeks before Christmas. Please make other arrangements, such as using a courier service.

FORWARDING SAMPLES TO OTHER LABORATORIES

An increasing number of requests are being received in Cardiff for tests involving a large number of individually rare disorders. A Specialist Genetic Testing Network has developed within the UK (UKGTN) and, where appropriate, samples for molecular and molecular cytogenetic tests not carried out in Cardiff are forwarded to other CPA Accredited laboratories within the UKGTN, particularly those within the local SCOBEC consortium (laboratories at Salisbury, Cambridge, Oxford, Bristol, Exeter and Cardiff, respectively).
TEST REPERTIOIRE OF CYTOGENETICS LABORATORY

CYTOGENETICS

Under the current NHS contract for patients resident in Wales, cytogenetic analysis is performed for the following clinical indications.

Blood sample indications
Peripheral blood is the tissue of choice for most postnatal cytogenetic investigations.

1. Physical features, such as dysmorphism, congenital anomalies and short stature, suggestive of an underlying chromosomal anomaly
2. Specific syndromes associated with chromosome anomalies, including microdeletion syndromes
3. Chromosome breakage syndromes
4. Ambiguous genitalia
5. Developmental delay / learning difficulties
6. Infertility
7. Gamete donor
8. Primary / secondary amenorrhea and premature ovarian failure / early menopause
9. Recurrent miscarriages
10. Previous child with congenital anomalies (chromosomal status unknown)

11. Family history of a chromosomal anomaly (e.g., parent of child / foetus with a chromosome abnormality)
12. Prenatal diagnosis follow-up

Bone marrow and leukaemic blood sample indications
1. Chronic leukaemia
2. Acute leukaemia
3. Myelodysplasia (confirmed cases)
4. Myeloproliferative disorders
5. Other haematological disorders
6. Lymphoma (with marrow involvement)

Amniotic fluid, CVS and foetal blood sample indications
1. Abnormal ultrasound scan (excluding soft markers)
2. Down screening risk of 1 in 150 and above.
3. Previous child/pregnancy with a chromosomal anomaly or with a congenital anomaly (chromosomal status unknown)
4. Family history of a chromosomal anomaly
5. Parent a carrier of a chromosomal anomaly/genetic condition
6. Determination of gender (for X-linked disorder)
7. Confirmation of CVS or amniotic fluid result
Fibroblast culture (from skin and other solid tissues) indications
1. Suspected mosaicism
2. Abnormal foetuses
3. Termination of pregnancy for chromosome abnormalities and anomalies detected on ultrasound scan
4. Perinatal, neonatal and other infant deaths, with suspected chromosomal cause

Molecular Cytogenetics

Fluorescence In Situ Hybridisation (FISH)
This involves the application of fluorescently labeled DNA probes to chromosomes. The FISH service complements routine karyotype analysis. By using chromosome-specific ‘paint’ probes, together with region-specific probes it is now possible to unequivocally identify all imbalances detected during conventional microscopic analysis. Furthermore, use of chromosomal region-specific probes allows laboratory confirmation of an increasing number of syndromic conditions involving microdeletions or duplications which are normally not visible. Sub-telomeric probes can also be used to detect small cryptic imbalances at the ends of the chromosomes. In addition, FISH can be applied to non-dividing, interphase cells in some circumstances, eliminating the need for cell culture and reducing the time required to generate a result. A number of FISH probes, including those that show oncogene fusion, and ‘break-apart’ probes, applicable to interphase and metaphase cells, are invaluable for the detection or confirmation of specific rearrangements in leukaemia and allied disorders.

Identification of visible imbalances
A very important application of FISH is the use of chromosomal painting, together with region specific probes (e.g., sub-telomeric / centromeric) to characterise additional or missing chromosomal segments not identifiable by conventional banding techniques. This has developed to become an essential part of our methodology that allows accurate delineation of structural chromosomal abnormalities and marker chromosomes detected during microscopic analysis. This increases the clinical utility of cytogenetic testing, improving the accuracy of information available for patient or family management, and is especially valuable in prenatal investigations.

Microdeletion detection
Commercially available region-specific probes, complemented by probes developed in-house are employed for the identification of chromosomal deletions and duplications suspected on clinical grounds and not detectable by conventional light microscope analysis. This technique allows unequivocal diagnosis of an increasing number of syndromes including:
- Cri du Chat syndrome
- Isolated lissencephaly sequence
- Kallman syndrome
- Miller-Dieker syndrome
• Prader-Willi / Angelman syndrome (only in exceptional circumstances as this is now a molecular test)
• Smith-Magenis syndrome
• Williams syndrome
• Wolf Hirschhorn syndrome
• 1p36 deletion syndrome
• 22q11 deletion / duplication / DiGeorge / Velocardiofacial syndrome
• 22q13 deletion syndrome

Although other clinically important microdeletion syndromes can now be confirmed by FISH analysis, not all probes are available for routine use in Cardiff. Should there be a need for a FISH analysis that we currently do not provide, clinical users of the service are encouraged to call Peter Thompson or Selwyn Roberts (telephone nos. 029 2074 4024 and 029 2074 2641, respectively) before forwarding the sample to the Laboratory.

Sub-telomere screening
A commercial set of 50 probes is employed to screen all telomeres to detect sub-telomeric imbalances in a test that is carried out on developmentally delayed patients, who satisfy certain other clinical criteria, and in whom no abnormality has been detected on conventional cytogenetic analysis. However, this test is now carried out only in exceptional circumstances as resources for this test has been transferred to array CGH testing, which is a significantly more powerful method for detecting small imbalances (see below). Specific sub-telomeric probes continue to be employed to identify additional or missing segments detected by conventional cytogenetic studies.

Malignancy diagnostics
An increasing number of FISH probes for specific abnormalities, including rearrangements involving oncogene activation/repression in leukaemia and allied disorders, are available. FISH studies are carried out using the BCR / ABL1 probe set on all Ph negative CML cases and a specific probe set (ATM and TP53) is employed on all CLL cases received. Other FISH probes will be used as appropriate in accordance with the ACC Best Practice Guidelines to detect or confirm specific abnormalities.

QF-PCR Rapid aneuploidy testing
Amniotic Fluid Samples QF-PCR
The Cytogenetics Laboratory for Wales has been supported by Health Commission Wales to provide rapid aneuploidy testing on amniotic fluid samples. We are now able to provide rapid aneuploidy testing by Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) on all amniotic fluid samples received. This service commenced on 16th January 2006. This development provides equity of access to rapid prenatal aneuploidy testing in Wales.

The autosomal multiplex analysis technique carried out routinely only detects trisomy 21, 18 and 13, and triploidy. However, in pregnancies where cystic hygroma has been identified on USS, a 45,X screen is also carried out using sex chromosome markers. In addition, the sex chromosome multiplex is employed to
determine foetal sex, where this is indicated.

Since about 20% of clinically significant abnormalities will not be detected by QF-PCR, cell culture and karyotyping continue to be carried out on all samples. Patients will, therefore, need to be appropriately counselled.

QF-PCR reports are available within 3 working days of receiving the samples in nearly all cases, and the majority will be reported more rapidly than this. Currently, about 95% of samples are reported within 2 working days of receipt of the sample in the Laboratory. Samples should arrive in the Laboratory by 3 pm for reporting on the next working day to be possible. In order to achieve the best turnaround time, it is also preferable to carry out amniocentesis procedures at the beginning of the week rather than towards the end, and, in particular, to avoid sending samples on Friday, otherwise results will not be available until the following week. Results are sent by facsimile with secure arrangements for sending and receiving the reports. All abnormal and ambiguous results in prenatal cases are also reported by telephone by a senior member of the scientific staff and, if necessary, the case will be discussed fully with the referring consultant.

Recent studies 1, 2 have shown QF-PCR is a very reliable and accurate test for non-mosaic trisomy 21, 18 and 13. However, about 3% of samples will fail to yield a QF-PCR result, primarily due to maternal blood contamination. In about 5% of cases, maternal, and occasionally paternal, blood samples will be required to interpret the results. 

As standard karyotyping continues to be carried out on all amniotic fluid samples received, it would assist us to maintain current reporting times on cell cultures if adequate volumes of amniotic fluid (1 ml for each week of gestation) are provided in all cases.


**Chorionic Villus Samples QF-PCR**

QF-PCR is carried out on all CVS samples. This replaces the Direct CVS microscopic karyotype analysis. As with amniotic fluid samples, CVS QF-PCR reports are available within 3 working days of receiving the samples in nearly all cases, and the majority are reported much more rapidly than this. Currently, about 97% of samples are reported within 2 working days of receipt of the sample in the Laboratory.

The autosomal multiplex analysis technique carried out routinely will only detect trisomy 21, 18 and 13, and triploidy. However, in pregnancies where cystic hygroma has been identified on USS, a 45,X screen is also carried out using sex chromosome markers. In addition, the sex chromosome multiplex is employed to determine foetal sex, where this is indicated. Reporting arrangements are the same as for amniotic fluid QF-PCR results.

CVS QF-PCR is a reliable test. Nevertheless, experience in UK laboratories has shown that discordance can occasionally occur.
between the results of CVS QF-PCR and karyotyping CVS cultures and foetal tissue, for the chromosomes tested. As is the case with CVS direct karyotype preparations, these discrepancies appear to be mainly false positive results due to confined placental mosaicism, but false negative results can also occur. Although these discrepancies are relatively rare, occurring with a frequency of about 1 in 2,000 samples, as with direct CVS karyotype results, we would advise caution. Clinicians should consider waiting for the CVS culture results before acting on an abnormal QF-PCR result, particularly if no foetal abnormality is evident on ultrasound scan.

In common with other UK laboratories, we now extract DNA for the CVS QF-PCR analysis from a small portion of the dissected, minced, trypsin-digested material produced from all available villi for CVS culture. This ensures that both mesenchymal and cytotrophoblast cells from more than one villus are sampled for PCR, thus minimising the problems caused by clonal differences between individual villi and cell types and maximising the chances of detecting a major clone. It is envisaged that the discrepancy rate will be lower using this approach.

A maternal blood sample in EDTA is now required with each chorionic villus sample. This enables the Laboratory to distinguish between maternal and foetal genotypes in cases of maternal cell contamination.

**Solid Tissues QF-PCR**  
QF-PCR is carried out on a sub-set of solid tissue samples where indicated. This includes confirmations of prenatally detected aneuploidies in foetal tissue, samples where culture growth is unlikely to succeed because of the age or nature of the specimen, and samples from foetuses and stillbirths with a specific diagnosis. QF-PCR replaces FISH ‘touch preparations’ in these situations.

**Blood Samples QF-PCR**  
QF-PCR is carried out on a sub-set of blood samples. This includes maternal, and occasionally paternal, blood samples to help interpret amniotic fluid and CVS QF-PCR results, and, foetal blood samples. QF-PCR is also carried out on blood samples from neonates in whom aneuploidy is suspected (autosomal multiplex) and neonates with ambiguous genitalia (sex chromosome multiplex).

**MLPA**  
MLPA (Multiplex Ligation-dependent Probe Amplification) analysis of DNA is carried out on non-urgent cases referred for Di-George/Velocardiofacial (VCFS) syndrome/22q11 deletion syndrome using the MRC-Holland SALSA DiGeorge P023B and/or P250 probe kits. The kits contain 28 reference probes which map to 7 other chromosomal regions, deletions of which have been associated with features of DiGeorge syndrome/VCFS, including 4q21->4q25, 4q34.2->4q35, 10p12->p15, 8p23.1, 17p13, 18q21.33 and 22q13. The kits are a very efficient and accurate means of detecting deletions and duplications in the 22q11 region.

**Microarrays**
As part of ongoing research and development, the technique of array comparative genomic hybridisation (CGH), has also been established locally. This technique provides a very powerful copy number genetic analysis at very high resolution. This technology has been successfully piloted with commercially produced BAC and oligo microarray platforms. It is a test that is currently carried out only on developmentally delayed patients, who satisfy certain other clinical criteria, and in whom no abnormality has been detected on conventional cytogenetic analysis. Array CGH is currently available only for a limited number of patients referred by Clinical Geneticists. This technology, if fully commissioned, has the potential to dramatically improve the resolution and clinical utility of cytogenetic analysis in a range of clinical settings.
REPORTING TIMES, SUCCESS RATES AND OTHER REPORTING ISSUES

SAMPLE REPORTING TIMES

Sample reporting times vary to some extent from year to year with fluctuations in workload and staffing levels, but priority is given to urgent samples, such as prenatal samples and samples from newborn infants. The average reporting times for the 2010 calendar year is shown in the Table below.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average Reporting Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>QF-PCR amniotic fluid</td>
<td>1.3 wd</td>
</tr>
<tr>
<td>QF-PCR CVS</td>
<td>1.4 wd</td>
</tr>
<tr>
<td>Amniotic fluid cultures</td>
<td>11.1</td>
</tr>
<tr>
<td>CVS culture</td>
<td>10.8</td>
</tr>
<tr>
<td>Urgent bloods</td>
<td>6.5</td>
</tr>
<tr>
<td>Non-urgent bloods</td>
<td>26.5</td>
</tr>
<tr>
<td>Solid tissues</td>
<td>25.5</td>
</tr>
<tr>
<td>Urgent bone marrows</td>
<td>7.0</td>
</tr>
<tr>
<td>Non-urgent bone marrows</td>
<td>16.9</td>
</tr>
</tbody>
</table>

95% of QF-PCR prenatal test results were reported within 2 working days (wd) and 71% were reported on the working day following the receipt of the sample in the Laboratory.

The majority of prenatal cultured amniotic fluid samples were reported within 12 days and some considerably earlier than this. 99% of amniotic fluid and chorionic villus sample cultures were reported within 14 days.

All prenatal results are ‘faxed’ to the TASCOs/ANC sisters, using ‘safe haven’ facsimile numbers, immediately they are available. All abnormal and ambiguous results in prenatal cases are also reported by telephone by a senior member of the scientific staff and, if necessary, the case will be discussed fully with the referring consultant.

Over 99% of urgent blood samples were reported within 10 days. Very urgent blood samples can be reported within 24 hours by QF-PCR or within 48 hours by karyotyping. In the latter cases a provisional result may be given because the preparations in 48-hour cultures are often inferior to those in 72-hour cultures. More subtle changes may therefore only be visible in the higher resolution analysis possible in 72-hour cultures, and these may take a day or two longer to report. All abnormal urgent results will generally be reported by telephone in the first instance by a senior member of the scientific staff.
81% of non-urgent blood samples were reported within 28 days.

82% of solid tissue samples were reported within 28 days.

99% of urgent bone marrow samples were reported within 14 days. Very urgent samples can be reported within 24 hours.

95% of non-urgent bone marrow samples were reported within 21 days.

All written reports provide a full interpretation of the findings. Consultant Grade or Principal Grade clinical scientists authorise all reports and are available in the Laboratory during normal working hours to give advice to referring clinicians and to interpret results.

**CULTURE SUCCESS RATES**

The Cytogenetics Laboratory meets the ACC Best Practice Guidance for culture success rates. In the calendar year 2010, 99.2% of amniotic fluid samples and 100% of CVS samples were successfully cultured allowing a result to be issued. In addition, 98.9% of urgent blood samples, 99.4% of non-urgent blood samples, 91.9% of urgent bone marrows, 94.2% of non-urgent bone marrows and 80.4% of solid tissues were successfully reported.

**AUDIT AND QUALITY ASSURANCE**

**Prenatal outcome**

Please complete the Pregnancy Outcome Form, which is sent out with the report, for pregnancies where prenatal cytogenetic investigations have been carried out. This enables us and Antenatal Screening Wales to audit the cytogenetic prenatal testing service and aspects of clinical practice, and provides ongoing quality assurance in respect of the accuracy of the test results.

**Haematological / malignancy follow-up**

Please complete the Malignancy Follow-up Form, which is sent out with the report, to inform us of the final diagnosis or to confirm the original diagnosis in cases where bone marrow or blood samples have been taken for haemato-oncological cytogenetic investigations. This enables us to audit our abnormality rates in specific confirmed disorders.
POSSIBLE REPORTING COMPLICATIONS IN CYTOGENETICS

PRENATAL SAMPLES

Amniotic fluid and CVS QF-PCR samples

- Approximately 20% of clinically significant abnormalities will not be detected by QF-PCR but will be detected by conventional karyotyping, which will normally be carried out on all samples. Patients will, therefore, need to be appropriately counselled.
- About 3% of samples will fail to yield a QF-PCR result, nearly all due to maternal blood/cell contamination.
- In about 5% of cases maternal, and occasionally paternal, blood samples will be required to interpret equivocal results, caused primarily by maternal cell contamination, and in a small number of cases, by other problems, such as somatic microsatellite mutation.
- Discordance of QF-PCR results with CVS culture karyotypes, in respect of the chromosomes tested, may occasionally occur (approximately 1 in 2,000 samples); these are mainly due to false positive QF-PCR results, reflecting confined placental mosaicism, but false negative results may also occur.

Amniotic fluid culture samples

- A small number of samples (about 1 in 300) fail to produce adequate cell growth, so chromosome analysis is not possible. A repeat amniocentesis or cordocentesis may then need to be considered.
- An abnormal chromosome complement may be present in only a proportion of the cells of the foetus (mosaicism). The abnormal cell line may remain undetected by chance because of its low frequency or because of preferential growth of the normal cell line.
- A chromosome abnormality may be found in 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 it may be indicative of an abnormal cell line in the foetus. A senior member of staff will offer to discuss these cases individually with referring consultants and a repeat amniocentesis or a cordocentesis may then need to be considered. Genetic counselling will be available to the couple in such cases.
- Vary 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. In such cases, QF-PCR analysis may alert staff to the inconsistency between the karyotypic sex and the amelogenin genotype, if foetal male cells were present in the original sample.
- A foetal chromosomal rearrangement or supernumerary chromosome may be detected. Parental chromosomes may need to be analysed to assess the
clinical significance of the finding. In some cases it may not be possible to unequivocally determine whether the anomaly in the foetal karyotype will cause any clinical problems in the child. Genetic counselling will be available to the couple in such cases.

- A chromosome abnormality that is usually associated with only relatively minor clinical problems may be detected. Again, genetic counselling will be available to the couple in such cases.

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

- Microdeletions will generally not be detected in routine cultures. The detection of microdeletions requires high resolution analysis, which is not usually possible in amniotic fluid cultures, and / or the use of syndrome-specific FISH probes, the application of which is dependent on a prior clinical diagnosis.

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

**Chorionic villus samples (CVS)**

In addition to the above problems that can occur also in CVS samples, CVS samples may have other more specific reporting problems.

- Confined placental mosaicism may be present, which may result in the need for further invasive tests. Genetic counselling will be available to couples in such cases.

- Trisomy correction in the placenta may result in a false negative result or undetected uniparental disomy (UPD) of an imprinted chromosome.

- False positive results, which are more common in direct preparations of villi, QF-PCR investigations, and mosaic karyotypes, may occur.

**POSTNATAL SAMPLES**

**Blood samples**

- An abnormal chromosome complement may be present in only a proportion of the cells of the patient (mosaicism). The abnormal cell line may remain undetected by chance because of its low frequency.

- Microdeletions will often not be detected in routine cultures unless a specific microdeletion syndrome is suspected. The detection of microdeletions usually requires high resolution analysis, and application of syndrome-specific FISH probes.

- Other small imbalances and rearrangements may not always be detectable, because of the subtlety of the changes, or because they may be invisible using conventional techniques.

**Solid tissue samples**
• Some samples, particularly post-mortem samples, may fail to produce adequate cell growth, so that chromosome analysis may not possible. QF-PCR may be carried out on these samples, but this technique will detect copy number changes only of chromosomes 13, 18 and 21 (and X and Y if the sex chromosome multiplex is employed).

• A chromosome abnormality may be found in a proportion of cells from one or more cultures initiated from the sample. This may be a cultural artefact (pseudomosaicism), or, in products of conception, may result from the growth of extra-embryonic cells, but it may also be indicative of an abnormal cell line in the foetus / infant / patient.

• In cultures initiated from placental tissue and products of conception, growth of maternal cells in the sample may result in the analysis indicating a normal female cell line which is not indicative of the foetal chromosome constitution.
# USEFUL CONTACT NUMBERS

<table>
<thead>
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