TEST REPERTOIRE 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 amenorrhoea 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 16\textsuperscript{th} 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 cytотrophoblast 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->q25, 4q34.2->q35, 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.