

Cytogenetic Guidelines and Quality Assurance

A common European framework for quality assessment for constitutional and acquired cytogenetic investigations.



E.C.A. Permanent Working Group for Cytogenetics and Society

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GUIDELINES

1. INTRODUCTION

1.1 BACKGROUND

The Permanent Working Group “Cytogenetics and Society” of the European Cytogeneticists Association (E.C.A.) prepared these guidelines as a quality framework for cytogenetic laboratories in Europe in collaboration with EU sponsored Network of Excellence, ‘Eurogentest’ workpackage 1.4 (external quality assessment in cytogenetics). It is hoped that this document will lead to the establishment of common guidelines and standards that can be used as a reference manual in all European countries. This is particularly applicable to those countries without national guidelines, as they aim to achieve and

maintain high standards. The adoption of this document by E.C.A. will facilitate this process.

These guidelines are intended to assist in the development of national standards. Cytogenetic practises and regulations differ throughout Europe so in some instances these guidelines may not be in accordance with national/federal laws and regulations. In such cases, those regulations already form the basis upon which the national standards operate.

These guidelines take into account the existing quality assessment (QA) schemes, good laboratory practice (GLP) documents, accreditation procedures and protocols from different countries, as well as international policy documents. This document includes aspects of quality control and assurance for most of the routine

methods currently employed by cytogenetic laboratories. The following standards should be considered as minimum acceptable criteria, and therefore, any laboratory consistently operating below the minimum standard may be in danger of failing to maintain a quality service and satisfactory performance over an extended period of time. They should also be seen as guidance for certification and/or accreditation of cytogenetic laboratories.

In view of rapidly changing practices and technology, the guidelines will be revised regularly by the Working Group. The formation of European external quality assessment (EQA) network is also strongly endorsed. As some genetic tests could be performed with a variety of technologies any EQA programme must take this into account. Such an example could be the analysis of Prader-Willi syndrome in which the genetic analysis would be performed more accurately using a molecular genetic technique, than by cytogenetic analysis. Similarly, when looking for small deletions/duplications FISH analysis or molecular genetic techniques may be more appropriate to detect the abnormality than routine chromosomal analysis. Both cytogenetic services and cytogenetic EQA-programmes must therefore keep up to date with advancing technologies and in some cases this will involve a shift from a cytogenetic to a molecular genetic application.

At the end of this document is attached a list of national and international guidelines and policy documents as well as the other documents consulted in preparing these guidelines. This list is not exhaustive and as this is a rapidly changing area in genetics, the authors recommend that individuals working in this field keep abreast of the current literature and guidelines.

1.2 GENETIC COUNSELLING

The human genome is a fundamental element of personal and familial identity. Unlike other medical analysis, genetic tests, including cytogenetic studies, have broader implications on a psychological, social and reproductive level. Therefore, a vital component in constitutional cytogenetic testing must be referred by a medical doctor, nurse or a senior scientist trained in the genetics field in order to ensure appropriate expert counselling before and after testing. All genetic testing should be done with informed consent.

2. STAFF

There are different legislations, structures and traditions in organising cytogenetic laboratories in Europe. In recognising these differences, the managing director may or may not be trained/ specialised in Cytogenetics or have the management skills for the day to day running of a cytogenetic laboratory without a skilled supervisor. Consequently, the management of a laboratory can vary substantially. The following staff structure can therefore only address the skills required for those involved in the daily management of a cytogenetic laboratory.

2.1 DIRECTOR/MANAGER/(LABORATORY SUPERVISOR

A senior physician or senior scientist, with appropriate qualifications, should be responsible for the overall day to day running and control of the laboratory as well as responding to enquiries from clinicians, nurses or scientists. The laboratory supervisors must have adequate qualifications, education and experience for their position. The minimum qualifications are as follows:

- M.D. with specialisation in Genetics and Cytogenetics or
- Ph.D. with specialisation in Genetics and Cytogenetics or
- Degree or B.Sc. or M.Sc. with specialisation in Genetics and Cytogenetics or
- State registration with specialisation in Genetics and Cytogenetics

The number of years experience may depend on national regulations. Moreover, some countries may require additional professional qualifications.

2.2 DIAGNOSTIC WORK SUPERVISOR /SECTION HEAD

A senior scientist or senior physician, with appropriate qualifications and experience relevant to the laboratory's operations, directly supervises all the diagnostic work in the cytogenetic laboratory.

The minimum qualifications are as follows:

- Degree or B.Sc. or M.Sc. with specialisation in Genetics and Cytogenetics or
- State registration with specialisation in Genetics and Cytogenetics

Troubleshooting in cytogenetics (constitutional, aquired, or FISH) requires a person with specialised training and experience.

2.3 TECHNICAL STAFF

Staff members should have adequate education for the type of investigation they are performing. There should be evidence that less qualified staff are supervised by an appropriately qualified person.

2.4 TRAINEE STAFF

All trainee staff should follow a programme of training with a designated supervisor. There should be procedures in place to determine when a trainee is competent at a given technique /process.

2.5 ANCILLARY STAFF

Ancillary staff may perform clerical, cleaning, sterilisation and/or photographic work, although this may be included in the workload of technical staff.

2.6 ADMINISTRATIVE STAFF

Administrative staff, in addition to administrative duties, may also prepare cytogenetic reports, storage and retrieval of cytogenetic records and general enquiries to the department.

2.7 MEDICAL COLLABORATION

The laboratory should have access to medical expertise on regular basis. A clinical consultant should be available within a time scale appropriate to the urgency of any foreseeable clinical situation.

Senior clinical and laboratory specialists should have sufficient interdisciplinary training to ensure adequate working knowledge of each other's speciality.

2.8 SCIENTIFIC COLLABORATION

The laboratory should encourage research and scientific collaboration. For instance, if a laboratory is to generate and label its own FISH probes, an appropriate molecular biology trained staff member is required. If the individual is not employed by the department he/she should be available for advice during working hours.

3. WORKLOAD RECOMMENDATIONS

There will be considerable variation among staff members in their speed of analysis and the number of specimens processed, depending on the individual and also their other duties. Moreover the workload is influenced by the degree of automation, complexity of analysis involved and whether or not photographic work is necessary. The number of staff should be sufficient to ensure that no unnecessary delays occur in the processing of samples.

Taking all this into account, an average annual workload for a member of staff undertaking cytogenetic analysis, the following workload is expected (Ancillary and administrative staff are additional to the laboratory staff and are not included in this workload):

- 250-350 lymphocyte samples; or
- 250-350 prenatal samples; or
- 250-350 solid tissues; or
- 150-250 haematological samples; or
- 100-200 solid tumour samples; or
- 400-500 Metaphase/Interphase FISH tests; or
- 150-220 specialised FISH tests e.g. multiple sub-telomere

Obviously the workload will vary depending on the complexity and weighting of the different tissues within the laboratory e.g. in laboratories where a more complex or technically difficult oncology or FISH specimens predominate a reduced workload is appropriate.

Sufficient time should be allocated to developmental work and continuous professional education (CPE/CPD) of staff.

Once a technique has been established, to maintain expertise, a laboratory should process a minimum of 100 samples per year in a given cytogenetic field (prenatal, postnatal, acquired, or oncology). Otherwise it is recommended that samples be directed to another laboratory. To maintain staff competence a laboratory is recommended to process no less than 500 samples annually (including all sample types).

At least two diagnostic work supervisors, in addition to the Director of laboratory, are necessary in a diagnostic service laboratory in order to ensure adequate checking of results, continuity of service during absences or vacations and to cope with variation in workload.

4. LABORATORY PROCEDURES

4.1 GENERAL

The work location or work environment should be suitable for laboratory work, and have appropriate security to avoid unauthorised access to the laboratory. The work environment should also ensure minimal work-related injury to employees and visitors.

Lack of space or inappropriate equipment must not be a limiting factor for quality in culture or analysis.

4.1.1 REFERRALS

See Appendix 1 for indications for referral to a cytogenetic laboratory. The laboratory should have policies for onward referral where cases require specialised expertise not provided locally e.g. chromosome breakage analysis, molecular genetic testing.

4.1.2 STANDARD OPERATIONAL PROCEDURES (SOPs)

Standard operational procedures, for techniques or use of equipment, should exist for all operational procedures in the laboratory. They should be written in a language understandable for the staff. SOP's should be updated annually. Obsolete versions of SOPs should be kept for at least 5 years. It is the responsibility of the laboratory director to ensure that all staff are appropriately trained, and have knowledge about and understand the standard operating procedures.

4.2 EQUIPMENT AND FACILITIES

Essential equipment should be serviced annually. All equipment and facilities in the laboratory should fulfil the requirements for the European Community (CE approved). Council Directive 93/68/EEC: 1993

To minimise equipment failure, all essential equipment should be duplicated (i.e. two incubators, two centrifuges, etc.). If any essential equipment is not duplicated for any reason, the laboratory should have a written "crash plan" on how to overcome failure affecting the laboratory work.

4.2.1 SAFETY CABINETS

All fresh cytogenetic samples are at risk of carrying dangerous pathogens e.g. Hepatitis B positive blood samples. Appropriate safety cabinets should be used for the containment of biological material, see the EC Directive (93/88/EEC). Horizontal laminar flow cabinets should be avoided as they offer no protection for the worker. Many countries have National regulations for the protection of workers, samples and the environment. If no National regulations exist it is recommended to consult documents as:- EC Directive (93/88/EEC), HSC, Advisory Committee on Dangerous Pathogens, The management and design and operation of microbial containment laboratories (ISBN 0717620344) or ZKBS advisory committee in Germany.

4.2.2 INCUBATORS

All incubators and other critical equipment should be fitted with an alarm or an override system to protect against malfunction of temperature and CO₂ (where used) controls. It is recommended that centrally monitored alarm systems are available.

Laboratories performing prenatal analyses should possess at least two incubators for splitting of prenatal specimens. It is also recommended that prenatal and non-prenatal cultures are incubated separately to minimise the risk of microbial cross-contamination.

4.2.3 IMAGE CAPTURE SYSTEMS

To maintain a high quality service provision all image analysis systems should be maintained regularly with software upgrades.

The number of image processing systems should not be a limiting factor in specimen analysis. When using image analysis systems, one part of the analysis process, either the initial analysis or the checking, should be completed using a microscope to ensure small markers or additional chromosomes have not been overlooked.

To avoid unnecessary delays due to image systems faults/failure, a service agreement is highly recommended.

4.3 TECHNICAL ASPECTS OF CYTOGENETICS

4.3.1 CELL CULTURES

Duplicated or independently established cultures, where possible, are recommended for all types of cultured specimens.

4.3.2 BANDING

All karyotyping should be carried out using a banding technique. In no cases, except some chromosome breakage syndromes, should a report be issued without cells having been subjected to full analysis of the banding pattern for the whole chromosome complement.

The ISCN defines five levels of banding. This can be used as a guide for establishing the degree of resolution required in producing a result. Several useful methods have been developed to help assess banding quality. Some countries e.g. Germany and the UK use an alternative approach that designates a quality score representing which chromosome bands are visible at 250 (QAS 2), 350 (QAS 4) and 550 (QAS 6) bphs resolution. More information can be found on the ACC (www.cytogenetics.org.uk under info) and BVDH (www.gfhev.de/en/membership/ under quality management) websites.

Numerical and structural abnormalities have to be excluded at a banding level appropriate to the referral reason. One or more objective and reproducible method(s) must be used to assess banding resolution and must be described in the laboratory protocol book or User Guide.

A standardised method for assessing banding quality should be used, with an agreed minimum standard that may vary depending on the reason for referral. Full analysis must be completed to the satisfaction of the supervisor that numerical and structural abnormalities have been excluded to a level appropriate for the referral reason. Specific standards for resolution should be appropriate to the case and the type of tissue studied. The 400 bphs (QAS 4) level is the minimum

level of resolution for studies to establish common aneuploidies in constitutional cytogenetics. A 550 bphs (QAS 6) level should be the minimum standard for referrals of mental retardation, birth defects, dysmorphic children or couples with recurrent pregnancy loss.

Where it is not possible to achieve the minimum quality for the referral reason, and no clinically significant abnormality is detected, the report should be suitably qualified whilst not encouraging repeat invasive procedures when these are NOT clinically justified.

4.4 ANALYSIS

4.4.1 KARYOTYPING/ CHROMOSOME ANALYSIS

In general, a minimum of 5 cells should be fully analysed for constitutional analysis and 10 cells for a haematological analysis. If metaphase analysis involves a comparison of every set of homologues (including X & Y), band by band, then a minimum of 3 metaphases can be fully analysed. If one of the homologue pair is involved in an overlap with another chromosome the pair of homologues should be independently scored to ensure there is no structural rearrangement.

Additional cells may be counted depending on laboratory policy. An extended analysis and/or cell count is warranted when mosaicism is clinically indicated or suspected. The laboratory should have written protocol for the analysis criteria.

Hyper- and hypodiploid and polyploid cells should be fully analysed in constitutional and haematological analysis.

All cases should have an image or a slide stored for later review (see section 11.12.1).

Refer to the current ISCN for the definition of a clonal abnormality.

4.4.2 CHECKING

Checking of all cases by a second qualified cytogeneticist is essential. A senior supervisor or an experienced cytogeneticist should check the analysis.

4.5 INTRODUCTION OF NEW LABORATORY PROCEDURES

A laboratory should, when starting any new diagnostic service, have a protocol for training staff and testing new equipment so patients are not at risk from inappropriate handling of equipment/slides etc. One way of doing this is to divide the samples, and send half to an experienced laboratory until the necessary level of competence is achieved. Validation and SOPs of these procedures is required.

4.5.1 USE OF MOLECULAR TECHNIQUES

When molecular genetic techniques are more sensitive than conventional cytogenetics, they should be used once the method has been validated, (e.g. for Angelman or Prader Willi syndrome, other microdeletion syndromes, Fragile X syndrome, etc). This could result in onward referral of cases if a laboratory is unable to undertake such analysis. Exclusion of other

chromosome abnormalities may still be required in most cases.

5. SPECIFIC CHROMOSOMAL ANALYSIS

5.1 PRENATAL

5.1.1 AMINOCYTE CULTURES

To minimise the risk of contamination, or culture loss due to incubator failure, duplicate cultures should preferably be handled separately, kept in separate incubators, if possible, running on a different electrical circuits. Prenatal cultures should be maintained with two different cell culture media, or with different batches of the same cell culture media and other reagents. The possibility of maternal cell contamination, pseudomosaicism, true mosaicism and *in vitro* aberrations must be recognised and the system of culture and analysis used designed to detect and differentiate these problems.

Harvesting or subculturing of all cell cultures from an individual sample together should be avoided.

If possible back up cultures should be kept until the final report is written.

Facilities should be available for freezing viable cells, e.g. for unresolved cases of abnormal foetal pathology.

5.1.2 CHORIONIC VILLI CULTURES

Before a CVS sample is cultured it must be dissected and maternal decidua separated from the villus to reduce the chance of maternal cell contamination. It should be clear from the referral form whether the sample has been dissected or not prior to its arrival in the laboratory. If an initial cytogenetic diagnosis is made on short-term preparations, a long term culture should be available for confirmation, in order to minimise problems of interpretation (Euromic 1997, ACC Collaborative study, 1994). Analysis solely on short-term incubation preparations (direct preparations) is not recommended (Euromic 1997, ACC Collaborative study, 1994). If the sample is of an inadequate size for both short and long term cultures, analysis from a long term culture is recommended.

5.1.3 FOETAL BLOOD CULTURES

The foetal blood sample should be checked to ensure it is not mixed with maternal blood, and originates only from the foetus. Several haematological methods are available. (Alkaline Phosphatase, Kleinhauer, Coulter counter sizing). Both foetal blood and amniotic fluid samples should be analysed unless there is a valid reason not to do so e.g. abnormal foetal blood result and pregnancy terminated.

5.1.4 MOSAICISM IN PRENATAL STUDIES

Two or three cultures should be set up for each sample. Analysis of a second or third culture is essential in cases of suspected mosaicism or pseudomosaicism e.g. trisomy 2 or where the abnormality is not consistent with continued fetal development (see Hsu et al., 1996, 1997). In general, if the same abnormality is present in two independent cultures, mosaicism is confirmed.

For *in situ* preparations, analysing cells from one cell culture may be sufficient if not all from the same

colony. However, it is recommended that at least two independent cultures are established to be able to rule out pseudomosaicism. When sufficient colonies are available, no more than two cells should be counted and analysed from a single colony (except when excluding a single cell anomaly). If colonies are insufficient for this to be achieved, a comment should be made in the report. It is unreasonable to expect all cases of true foetal chromosome mosaicism or small structural rearrangements to be detected by a routine level of analysis.

A written procedure for delineating different types of mosaicism should be drawn up for guidance within the laboratory. Individual cases can require careful assessment and discussion and the number of cells counted and analysed may exceed the minimum.

In an amniotic fluid culture, detection of a mosaicism must be followed up by extensive examination of cells from an independent culture, or from independent colonies. Failure to confirm the abnormal cell line provides reassurance of a normal pregnancy but, depending on chromosomes involved and the nature of the abnormality, supplementary investigations may be appropriate (see Hsu et al., 1996, 1997). To facilitate the elucidation of mosaicism and *in vitro* abnormalities, the independent colony method is recommended. In CVS, the significance of mosaicism may depend on the distribution of the abnormality amongst different cell types in direct and cultured preparations, and the chromosome/s involved (Euromic 1997, ACC Collaborative study, 1994).

The possibility of foetal uniparental disomy in some cases cannot be ignored, and additional tests may be required to resolve uncertainty. UPD studies should be considered where there is mosaicism or confirmed placental mosaicism involving chromosomes 7, 11, 14 & 15 and in homologous and non-homologous Robertsonian translocations involving 14 & 15, and markers chromosomes of chromosome origin 7, 11, 14 & 15 (Kotzot 2002; Robinson et al., 1996; ACC Prenatal Guidelines, 2005).

5.2 POSTNATAL

5.2.1 POSTNATAL CHROMOSOME ANALYSIS OF BLOOD AND SOLID TISSUE/ PRODUCTS OF CONCEPTION.

See ANALYSIS (Section 4.4.1). Extended analysis includes analysing a minimum of 30 cells is recommended when clinically relevant mosaicism is suspected (giving appropriate confidence limits using Hook's tables for lymphocyte cultures, Hook 1977). FISH analysis may be the most appropriate method of confirming suspected numerical mosaicism if a suitable probe is available. In some instances more than one tissue type should be investigated e.g. Pallister-Killian syndrome or trisomy 8 mosaicism. Referring clinicians must be made aware that it is not possible to reliably exclude mosaicism from any analysis.

5.2.2 MOSAICISM

In cases where mosaicism may be expected to be present (e.g. sex chromosomes abnormalities or chromosome breakage syndromes), the number of cells counted and scored should be sufficient to rule out mosaicism or clonality. An extended analysis is usually adequate (see 5.2.1). However, the laboratory should consider the common occurrence of age related sex chromosome losses and/or gains before reporting sex chromosome mosaicism (Guttenbach et al., 1995; Gardner and Sutherland, 2003). Laboratories should also be aware that the level of mosaicism may vary between tissues.

5.2.3 PRODUCTS OF CONCEPTION/FOLLOW UP SPECIMEN

Follow up of abnormal cases may form a part of internal quality control. However, if foetal morphology does not confirm the laboratory findings, foetal tissue samples should, where possible, be analysed.

5.2.4 CHROMOSOME INSTABILITY SYNDROMES

The rarity of chromosome instability syndromes and the interpretational problems associated with chromosome breakage syndromes requires that inexperienced laboratories refer such cases to laboratories with proven expertise. Classic breakage syndrome disorders include: Ataxia telangiectasia, Bloom syndrome, Fanconi anaemia, Nijmegen syndrome. Other syndromes involving defective DNA replication/repair (e.g. Cockayne syndrome and Xeroderma pigmentosum) are not amenable to cytogenetic methods of confirmation.

Clastogen studies should only be undertaken with appropriate negative control samples and, if available, positive control samples.

All control and test samples should be collected, processed, cultured and harvested in parallel.

Controls should be appropriately matched (e.g. sex, age etc.). The patient and control samples should be analysed blind.

Sufficient numbers of metaphases must be examined in order to ensure that any chromosomal damage detected is significant.

- **Bloom syndrome**

As some affected individuals have a population of cells with a normal SCE frequency, examination of 20 metaphases is advisable. The laboratory should have a record of the SCE frequencies found when the same methods are applied to a range of normal control samples.

- **Fanconi anaemia**

Diagnosis and exclusion should be made by analysis in cultures exposed to clastogenic agents. Sufficient cells must be examined to exclude the possibility of somatic mutation, which is common in Fanconi anaemia. Analysis of at least 50 but preferably 100 metaphases is recommended. The efficacy of the clastogen used should be checked against either an untreated control or SCE levels in treated samples.

- **Ataxia telangiectasia and Nijmegen syndrome**

The aberration frequency in irradiated cultures, scored from 50 to 100 metaphases, should be compared with normal control cultures. As some ataxia telangiectasia patients display an intermediate response to irradiation, screening of 50 banded metaphases for rearrangements, involving the T-cell antigen receptor loci on chromosomes 7 and 14, should also be carried out.

5.2.5 OTHER RARE SYNDROMES DETECTED BY CYTOGENETIC ANALYSIS

Despite recent advances in the understanding of the molecular basis of some disorders, cytogenetic studies are often the first step in making a diagnosis.

Sufficient numbers of metaphases must be examined in order to ensure that any chromosomal damage detected is significant.

- **Roberts syndrome**

Fifty block (Leishman/Giemsa stained) or C-banded metaphases should be scored for paired centromeres, centromeres puffing and tramline chromosomes. Fifty banded metaphases should be counted, for evidence of aneuploidy.

- **ICF syndrome**

Fifty banded metaphases should be scored for anomalies of the heterochromatic regions of chromosomes 1, 9 and 16 and for multi-branched configurations.

5.3 ONCOLOGY: LEUKAEMIAS AND SOLID TUMOURS

All laboratories offering a diagnostic service should be able to provide an analytical and interpretive service for a range of haematological disorders see Appendix 1. Referral can be at diagnosis, follow up after treatment, including transplantation, relapse/transformation or as part of a national or locally agreed trial.

5.3.1 BONE MARROW

In haematological and solid tumour cultures, the culture conditions should be optimised where possible by utilising direct, short term and synchronised cultures to improve the mitotic index. Laboratories should be aware that culture times may affect the detection of an abnormal clone. When B- or T-cell lymphoproliferative disorders are suspected, suitable mitogens should be added to additional cultures.

5.3.2 SOLID TUMOUR

Solid tumour cultures may require both multiple cultures and longer incubation (>72hours). It is recommended that the laboratory has previous experience in the tissue culture of various cell types before setting this up as a diagnostic service.

5.3.3 HAEMATOLOGICAL CHROMOSOME ANALYSIS

Sufficient number of cells should be examined to detect the presence of clonal evolution. The quality of metaphases obtained from unstimulated blood and from bone marrow samples is generally poor, particularly in leukaemia. As normal cells with better chromosome morphology may be present, it is important to analyse cells of varying quality in order to maximise the likelihood of detecting a clone. Abnormal cells are often those of poorer quality and

sufficient cells should be analysed to establish the clonality of the abnormality (see ISCN for definition of clonality).

There is a high possibility of an abnormality being present in either a few cells or the presence of several subclones. When a normal karyotype is found, it is preferable that a minimum of 10 cells are fully analysed and a further 10 are screened for abnormal chromosomes for diagnostic samples, referral at relapse or transformation. If a sample yields fewer than twenty normal cells, the report should be suitably qualified.

For referrals where cytogenetic follow-up after treatment/remission is required the following analysis is recommended:

- If a normal result was obtained at diagnosis, further analysis is usually not appropriate.
- If abnormal result was obtained at diagnosis: a minimum of 20 metaphases should be scored for the relevant anomaly. In some instances, FISH may be appropriate for follow-up studies.
- For post-transplantation samples, a minimum of 30 metaphases should be scored for the presence or absence of the marker used to differentiate between donor and recipient cells e.g. the Y chromosome in mixed sex transplants. FISH may be more appropriate here also.

N.B. Definition of Scoring - To check for the presence or absence of a particular karyotypic feature in a number of cells

5.3.4 ONCOLOGY CHROMOSOME ANALYSIS

Adequate numbers of metaphases of **varying quality** should be analysed or examined before the report of a normal karyotype or of the existence of an abnormal clone is given. If a sample yields fewer than ten normal cells, the report should be suitably qualified.

Reporting and interpreting the results of tumour work is a specialised area, where close co-operation between the laboratory and the referring histopathologist is vital.

5.4 CGH AND MICROARRAY TECHNOLOGY

These methods are still considered to be experimental. However, any laboratory using these techniques in their clinical work should introduce SOPs. Clinical samples analysed according to these techniques should be treated as routine samples once an internal validation of the test has been established and handled according to appropriate laboratory guidelines.

6. FLUORESCENCE IN-SITU HYBRIDISATION (FISH)

6.1 GENERAL

Interphase and metaphase FISH, either as a single probe analysis, or using multiple chromosome probes, can give reliable results in different clinical situations. It should be noted that there may be variation in probe signals both between slides (depending on age, quality, etc. of metaphase spreads) and within a slide. Where a deletion or a rearrangement is suspected, the signal on the normal chromosome is the best control

of hybridisation efficiency and control probe also provides an internal control for the efficiency of the FISH procedure.

Depending on the sensitivity and specificity of the probe and on the number of cells scored, the possibility of mosaicism should be considered, and comments made where appropriate.

When hybridisation is not optimal, the test should be repeated. When a deletion or another rearrangement is suspected, the results must be confirmed with at least one other probe.

Results should preferably be followed up by karyotype analysis. This is essential when there are discrepancies between the expected laboratory findings, and the clinical referral.

Before introducing interphase FISH as a diagnostic technique, staff need appropriate training on the type of samples to be analysed. Laboratories should set standards for classification of observations and interpretation of results.

6.2 EQUIPMENT, FACILITIES AND SAFETY

A dedicated work area should be available for FISH work.

Specialised equipment should include facilities for incubation of tubes at varying temperatures, micro-centrifuge, fluorescent microscope with appropriate filters and camera or image analysis system.

Fume cupboards should be installed to protect staff where hazardous chemicals, such as formamide, are used.

Laboratories that are making their own probes should ensure their procedures prevent DNA contamination.

6.3 REAGENTS

Any new batch of labelled probes, whether generated in-house or purchased commercially, requires validation concerning its' performance before being used diagnostically. This validation requires testing for:

- *Target specificity*: To test if the probe hybridises to the correct location - preferably on both normal and abnormal chromosomes demonstrating the specific aberration
- *Analytical sensitivity and specificity*: These involve assessment of the proportion of targets demonstrating a signal (sensitivity), and proportion of signal at the target site compared with other chromosome regions (specificity). For most commercially available probes, the supplier has usually established these parameters. Sensitivity and specificity must be high to avoid misdiagnosis.

Any validation data should be fully documented for later internal audit.

6.4 TECHNIQUES

6.4.1 WHOLE CHROMOSOME PAINTING

Commercially available paints are generally used as they are reliable. Care should be taken in interpreting breakpoint positions from FISH results, and it should be done in conjunction with banding studies.

It should be noted that the resolution of chromosome painting may vary between different paints. Small rearrangements may not be detected since whole

chromosome paints may not be uniformly dispersed across the full length of the target chromosome.

6.4.2 DETECTION OF MICRODELETION AND firmed by alternative methodologies (e.g., molecular analysis, densitometry).

6.4.3 INTERPHASE FISH

Extreme care needs to be taken in interpreting results. The signal in interphase cells can be variable, so large numbers of cells must be examined. For detection of minimal residual disease in neoplastic disorders a large number of cells must be analysed.

In prenatal diagnosis the presence of maternal contamination should be noted and recorded accordingly. It should be noted that interphase FISH analysis could only detect a subset of chromosome abnormalities and may not provide a complete result or may be misleading in the absence of conventional banded cytogenetic analysis.

Interphase FISH may be an adjunct test to assess levels of mosaicism or chimerism of cell lines with abnormalities previously established by standard chromosome analysis.

6.5 ANALYSIS

It is not recommended that FISH be used routinely to confirm cytogenetically visible abnormalities although it should be used to check uncertain variants of diagnostic or prognostic significance. It may also be appropriate to check apparently classical abnormalities in the context of an atypical presentation.

6.5.1 CONSTITUTIONAL

Locus-specific probes – 5 cells should be scored to confirm or exclude an abnormality.

Multiprobe analysis – Three cells per probe should be scored to confirm a normal signal pattern. Where an checker each, when an abnormality is detected but extended to 100 cells each before exclusion of the abnormality is suggested.

6.6 CHECKING

Preferably interphase FISH results should be independently scored by an appropriately trained person each who should examine 30-70% of the total of cells used for the analysis. If the two primary scores differ significantly then a third person (if necessary from another laboratory) should be called in to provide a resolution. This person should normally be informed of the previous scores.

For metaphase FISH the same process should be used as for checking conventional banding.

7. QF-PCR FOR RAPID PRENATAL DIAGNOSIS OF ANEUPLOIDY

This technique is a useful adjunct to prenatal diagnosis and is a more appropriate technique than FISH when dealing with large numbers of prenatal referrals. Internal validation is essential before using this technique.

SUBTELOMERIC REGION ANALYSIS

Commercially available kits are generally used in diagnostic laboratories. The number of cells scored needs to be commensurate with the sensitivity and specificity of the probe on the slide. If microduplication is suspected, results should preferably be con-

abnormal pattern is detected, confirmation is advisable.

Prenatal interphase screening for aneuploidy

- Signals should be counted in at least 30 cells for each probe set.

Interphase screening for mosaicism

- A minimum of 100 cells should be scored.

6.5.2 HEAMATOLOGY AND ONCOLOGY

The standard number of cells recommended for a FISH study is 100. However, it is recognised that an adequate positive result can often be obtained with fewer (particularly when expecting an all-or-nothing result, e.g. in CGL), and a suspicious finding may need more.

In all diagnostic FISH studies, a positive effort should be made to examine a few metaphase cells, if present, and not depend entirely on interphase nuclei. In normal metaphases this confirms that the correct probes were used and abnormal metaphases can be invaluable in interpreting unusual signal patterns.

Laboratories should be aware of the different types of FISH probes and their normal signal pattern e.g. break-apart probes and fusion probes. The limitations of the probe set should be documented, particularly if the analysis is done solely on interphase cells.

The use of FISH on paraffin wax sections in particular is an appropriate way to investigate specific rearrangements or gene amplification and has the advantage that tumour tissue can be directly screened. Analysis can be limited to ten cells scored by analyser and Close liaison with Molecular Genetics colleagues is necessary to establish this technique in a cytogenetic laboratory.

The limitations of QF-PCR in identifying chromosome abnormalities must be clearly known. It is recommended that testing for trisomies 13, 18 and 21 is carried out, whilst it is acceptable to test for sex chromosome aneuploidy in only a subset of referrals. QF-PCR analysis provides information only about the probe locus in question. It does not substitute for a complete chromosome analysis.

7.1 EQUIPMENT, FACILITIES AND SAFETY

The equipment should be regularly maintained and there should be procedures in place for the safe disposal of the waste. The genetic analyser used for the analysis of the STR products should be capable of 2 bp allele resolution and peak area/peak height quantification.

7.2 SAMPLE PREPARATION

For amniotic fluid, between 0.5 and 4 ml or 1/10 of the sample is recommended for QF-PCR analysis, as larger aliquots may compromise the karyotype

analysis. For chorionic villus samples, it is recommended that at least two chorionic villi taken from different regions of the biopsy should be processed to minimise the risk of misdiagnosis due to confined placental mosaicism.

A chelex-based method is recommended for DNA preparation as this does not require any tube-tube transfers. Home-made kits should be batch tested using at least a trisomy and a normal DNA sample to ensure consistent assay quality and trisomy diagnosis. A H₂O control must also be included in each PCR set-up to identify any DNA or PCR product contamination. Between 24-26 PCR cycles should be carried out as standard practice and a minimum of 4 markers for each chromosome tested to reduce the number of uninformative results. It is recommended that tri/tetra/penta/hexanucleotide repeat markers are used as these have fewer stutter peaks, although dinucleotide repeat markers are acceptable if few suitable markers are available within the tested region.

New markers not used previously for QF-PCR aneuploidy diagnosis should be validated by testing a minimum of 100 chromosomes, including aneuploid samples.

7.3 ANALYSIS

It is recommended that both the electrophoretogram and peak measurements, which can be transferred to a spreadsheet for convenience, are analysed. To ensure the quality of the data both minimum and maximum peak heights should be used. It is acceptable to fail individual markers if there are valid technical reasons such as bleedthrough between colours and electrophoretic spikes. It is acceptable to use peak height, peak area or both measurements to calculate allele ratios, although for results obtained from an automated sequencer it is recommended that peak area is used to minimise peak distortion due to widely-spaced alleles.

The area/height of the shorter length allele should be divided by that of the longer length allele and the normal range should not exceed 0.8-1.4.

To interpret a result as abnormal, at least two informative marker results should be consistent with a tri-allelic genotype, with all the other markers uninformative. It is unacceptable to interpret a result as abnormal if shown by only one marker. Confirmation of sample identity when a result is abnormal by repeat PCR of the DNA, re-extraction of samples, or mat blood analysis is recommended.

To interpret a result as normal at least two informative marker results consistent with a normal diallelic pattern are required, with all other markers uninformative. However, it is acceptable to report single marker results that have a normal diallelic pattern and all other markers uninformative as consistent with a normal chromosome complement, if the report states that the result is based on a single marker result and that this the result must be confirmed.

Where maternal cell contamination occurs, if allele ratios are inconclusive and/or the maternal genotype is

present at a high level it is recommended that the fetal genotype should not be interpreted.

7.4 REPORTING

It is recommended that the assumption that fetal material is tested, and the fact that mosaicism and small segment imbalance for chromosomes tested may not be detected should be included on the report, either in the main text or in a report rider. The locations of markers showing a triallelic result should be listed to define the trisomic region. It is acceptable to list markers on a normal report, although this should be done in a way that does not 'bury' the result.

It is acceptable to report normal QF-PCR results as 'consistent with a normal diploid complement for chromosomes 13, 18 and 21', 'an apparently normal complement of chromosomes 13, 18 and 21 was detected', 'no evidence of trisomy' or similar statement.

Abnormal reports should include an interpretative statement such as 'consistent with Down syndrome', 'associated with Down syndrome', 'indicative of Down syndrome' or 'predicted to be affected with Down syndrome'. It is important to be aware that the QF-PCR sex chromosome assay (Donaghue et al. 2003) is a highly stringent screen for monosomy X but NOT a diagnostic test. A result consistent with monosomy X, where all polymorphic markers have only a single allele peak and no Y sequences are present, may represent a normal female homozygous for all markers tested. Therefore such a result should either be confirmed using another technique, or reported as being consistent with monosomy X with the caveat that there remains a possibility that a normal female could give the same genotype.

The limitations of the chromosome analysis or FISH probe being used must be clearly known. FISH analysis provides information only about the probe locus in question. It does not substitute for a complete chromosome analysis.

Care must be taken in the interpretation of normal results from studies based on repeated sequence probes, due to rare individuals with small numbers of the target repeated sequence.

Interpretation of results requires supervision by an appropriately trained cytogeneticist or physician.

8. REPORTING

8.1 STANDARDISATION

It is the responsibility of the cytogeneticist to provide a clear and unambiguous description of the cytogenetic findings and an explanation of the clinical implications of the results. Reports should be issued in a standardised manner, clear to read for the non-specialist.

Handwritten alterations should never be made to the report. It is not necessary to include details of culture procedures, unless relevant, e.g. from direct or cultured CVS, direct or cultured tumour. Laboratory records should be auditable so that the individual cells and slide analysed can be traced back through to the culture reagents and receipt of sample. Analysis sheets

should include the resolution levels of the banding techniques used and details of any additional banding techniques used.

Reports should be issued in a standardised manner, clear to read for the non-specialist.

The report should include the following information:

- date of referral and date of report
- name of referring clinician
- laboratory identification
- patient identification using two different identifiers, i.e., name and birth date
- unique sample identifier
- reason for referral
- tissue examined
- clinical indication of test e.g. chromosome analysis or FISH
- Total number of cells counted and analysed for haematological disorders and interphase FISH
- the banding resolution level or a disclaimer if the quality is below the minimum standard for referral
- Karyotype in ISCN or summary statement if complex FISH result
- a comprehensive written description of any chromosome result/abnormality
- a written interpretation (that is understandable to a non-specialist)
- name and signature of the authorised person

The report of an ABNORMAL case should include the following in addition to the above:

- a clear written description of the abnormality, and whether the karyotype is balanced or unbalanced
- karyotype designation using correct ISCN nomenclature where practicable
- cell numbers should be given when mosaicism present
- the name of any associated syndrome/disease
- whether the cytogenetic result is consistent with the clinical findings, and/or an indication of the expected phenotype
- recommendations for genetic counselling when appropriate
- request for samples to confirm prenatal results as internal quality control. Postnatal confirmation of a prenatally diagnosed balanced rearrangement may help to ensure the karyotype record appears in the child's own notes

The report should include the above information unless National legislation states that is done by a different medical professional.

8.1.1 SUBSTANDARD ANALYSIS

In cases where the quality or level of the analysis fails to achieve agreed standards, the report should be qualified and explain the limitations of the results.

8.1.2 CHROMOSOMAL VARIANTS

Polymorphisms such as heterochromatin size, satellite size, fluorescent intensity or pericentric inversions of heterochromatin should, to avoid confusion for the

non-specialist, be excluded from the report and only documented in the patient's laboratory record.

Occasionally polymorphic variants need to be mentioned and their significance should be clearly indicated in the interpretative comments. e.g. donor vs. host bone marrow grafting.

8.1.3 MOSAICISM AND PSEUDOMOSAICISM

In general, reports should not mention mosaicism or pseudomosaicism, if it is apparently non-clonal or likely to be artefactual.

Deciding what constitutes a non-clonal aberration is not always easy, especially in cancer cytogenetics, so the application of general rules together with consideration of the clinical referral need to be kept in mind when reaching a decision. (For guidance see ISCN or EUCROMIC Quality Assessment Group, Eur. J. Hum. Genet. 1997, 5:342-350 or ACC collaborative study 1994).

8.1.4 MATERNAL CONTAMINATION

If maternal contamination is relevant to the interpretation of the report a comment should be made. It should always be noted in the internal report.

8.1.5 FISH REPORTS

Since FISH testing is now widely used in European laboratories and in accordance with professional custom, it is no longer necessary that FISH reports carry a disclaimer stating that the commercial probes have not been licensed for diagnostic use.

The report nomenclature should follow the latest of the ISCN edition where possible (see section 8.1). The report should contain a written description and interpretation of the result which clearly states the result is normal. The report should be in simple language so it can be clearly understood by the recipient/clinician.

Where strict use of ISCN nomenclature would make the report unwieldy, e.g., where large number of probes have been used, a summary comment may be given with appropriate comments in the report e.g. MLL rearrangement positive.

The probe name and source should be given for each case and any limitations of the probe should be clearly stated in the report.

The report should indicate whether a banded karyotype analysis has been undertaken or not. Where karyotype analysis has been undertaken, FISH results may be sent out prior to karyotype analysis but with the indication of their provisional nature (haematological where no metaphases are covered). This is of extreme importance with abnormal prenatal FISH results, where irreversible clinical actions could follow.

9. SUCCESS RATES

Success rates depend on sample quality on receipt and individual laboratory policies on processing sub-standard samples. Problems outside the control of the laboratory may result in periods during which the success rate may decrease significantly. Laboratories should audit their success rate so as to identify external and internal factors that are having an adverse

effect so that corrective action can be taken. These success figures are for samples received of adequate quality and should be achieved annually:

Amniotic fluid and long term CVS cultures	98%
Direct CVS	90%
Postnatal peripheral blood samples	98%
Foetal Blood samples	98%
Products of conception/foetal parts/skin biopsy	60%*
AML, ALL, CML, MDS, MPD	90%

*if the laboratory policy is to set up samples that have been delayed in transit or are macerated, the success rate would be expected to be lower.

For solid tumour samples it is not possible to set minimum standards due to the diversity of samples.

Each laboratory should keep records of the success rates for types of tissues where a diagnostic service is offered.

10. REPORTING TIME

Laboratory report times should be kept as short as possible. Laboratories reporting times should take into account the reason for referral and level of urgency. There should not be any delay in reporting the cytogenetic results due to insufficient staffing or administrative procedures. The report should be sent out no later than the next working day after completion of the analysis.

The laboratory should have a written policy for reporting time. Recommended report times for 90% of the referrals are given below:

amniotic fluid and long term CVS cultures	21 days
lymphocytes cultures	28 days
bone marrows and tumour cultures	21 days
solid tissue or solid tumour cultures	28 days
short term CVS cultures (directs)	7 days
urgent lymphocyte, bone marrow or cord blood cultures	7 days
prenatal aneuploidy FISH screening /QF-PCR	4 days

These report times includes all weekends and public holidays.

The decision to repeat a prenatal cell culture, due to primary growth failure, should be made no longer than after 14 days.

10.1 PROVISIONAL RESULTS

In general provisional results should be communicated verbally by a supervisor or qualified cytogeneticist, to the clinician with an indication that the analysis is provisional and include a comment on which types of abnormalities have not yet been excluded. When provisional results are given, a verified hardcopy must be issued.

The communication should be documented on the patient's laboratory record of the information given, to whom, by whom and the time and date. This also applies to final verbal results.

11. CLINICAL RECORDS AND STORAGE

11.1 RECORDS

In many countries, storage and filing of patient data is subject to National regulations. The following recommendations only apply where no such regulations exist.

11.1.1 RETENTION OF DOCUMENTATION

Filing should be undertaken in a logical and consistent manner and SOPs should exist on how to retrieve documentation and material. The file must contain a unique sample number and patient identification should include the full name and at least two of the following: date of birth, hospital identification number, social security number, address including postal code. The file must contain comprehensive information on tests performed e.g. probe name and source, the number of cells scored on the analysis sheet or image capture system

Each case should be stored so as to include sufficient banded material for reassessment if required. A minimum of 2 banded metaphases should be stored, either as slides, photographic images or as a digital image. Digital images should preferably be duplicated and, stored separately for long time storage.

11.2 SPECIMEN STORAGE

In many countries, storage of patient tissue is subject to National regulations. The following recommendations only apply where no such regulations exist.

If possible cultures or fixed cell suspension should be kept until the final report is written.

Relevant information to trace the processing of the case should be saved for at least 5 years, but preferably indefinitely, especially if abnormal.

Prenatal cell cultures with unique rearrangements should, if possible, be stored until after delivery. If the abnormality has not been fully identified, the cultured cells should be stored indefinitely in liquid nitrogen. Similarly, cancer cytogenetic suspensions should be stored to allow reanalysis later in the disease process. Relevant informed consent should be obtained.

11.2.1 STORAGE TIMES

All information necessary to trace the handling of the case should be stored for at least 2 years. Results, including computerised images or photo negatives, should if possible be stored indefinitely when abnormal. For each normal sample at least one, or preferably two images or slides should be stored in an

easily accessible way together with the referral and cytogenetic report for a minimum of 5 years. For metaphase FISH analysis a slide photograph or image of at least one informative cell should be kept for abnormal results and any interphase or metaphase FISH results that cannot be visualised using conventional chromosome analysis.

Where the request form contains clinical information not readily accessible in the patient's notes but used in the interpretation of test data, the request card or an electronic copy of it should be kept.

QUALITY ASSURANCE

12. GENERAL

The Quality System of each cytogenetic laboratory should be consistent with current national and international standards (ISO 15189:2003 or ISO 17025:2005).

13. ACCREDITATION

13.1 ACCREDITATION

This is a 'Procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks.' (ISO/IEC Guide 2 General terms and their definitions concerning standardization and related activity.)

Accreditation is peer group assessment that a laboratory's performance across the required standards is acceptable (it does not usually include an assessment of counselling process). The visiting peer group, preferably selected by an organisation outside the laboratory, should include persons with experience across the full repertoire of the laboratory. Accreditation should be with an EA recognised accreditation body.

Participation in an external quality assessment programme is one of many requirements for attaining an accredited status.

13.2 CERTIFICATION

Is a 'Procedure by which a third party gives written assurance that a product, process or service conforms to specific requirements' (ISO/IEC Guide 2 General terms and their definitions concerning standardization and related activity).

Certification is not the same as accreditation. Certification is based upon standards such as ISO9001:2000 which delineate the 'requirements for quality management systems' and are applicable to any activity. Accreditation systems are based on standards that in addition to 'requirements for quality systems' have so-called 'technical requirements' that relate to achieving competence in all aspects of laboratory activity. However, standards for quality management systems, such as ISO9001:2000 have a major impact upon the structure and content of standards used for laboratory accreditation. (Burnett D., A Practical Guide to Laboratory Accreditation in Laboratory Medicine, ACB Venture Publications 2002 London ISBN 0 902429 39 6.)

Certification only confirms that a laboratory adheres to the standards. It does not assess whether the laboratory's performance is acceptable. Certification of the counselling process may, where appropriate, be used as a complement to laboratory accreditation.

Some of the issues covered by Accreditation are given below. More detailed information can be found in the international standards documents (ISO 15189:2003 or ISO 17025:2000).

14. LABORATORY ORGANISATION AND MANAGEMENT

Laboratory management clearly demonstrates its commitment to fulfilling the need and requirements of its users by clearly defining the ways in which the laboratory is organised and managed. The laboratory should conform to ISO 15189/17025 standards or National equivalent (e.g. CPA). The laboratory should have a quality policy which sets quality objectives and has a commitment to achieve continual quality improvement. The laboratory management are responsible for the design, implementation, maintenance and improvement of the quality management system (see ISO 15189 or 17025 for further information).

Laboratory management should ensure there are procedures for personnel management including: staff recruitment and selection; staff orientation and induction; job descriptions and contracts; staff records; annual staff appraisals; staff meetings and communication; staff training and education; grievance procedures and staff disciplinary action. The laboratory management should ensure there are procedures for technical management including SOPs for all the pre- and post-analytical examination process.

The laboratory should have sufficient space allocated so that its' workload can be performed without compromising the quality of the work, quality control procedures, safety of personnel or patient care services.

15. QUALITY MANUAL

The quality manual describes the quality management system of the laboratory and arrangements for the implementation and maintenance of the quality service, including technical procedures. The roles, responsibilities and authority of all personnel shall be defined and procedures in place to control of process and quality records as well as control of clinical materials.

Each laboratory should have an appointed Quality Manager that oversees the establishment, implementation, maintenance and audit of the quality within a laboratory (internal and external)

16. DATA PROTECTION AND CONFIDENTIALITY

Confidentiality of genetic information is of utmost importance. Constitutional cytogenetic data may contain information that is of importance to individuals other than the person investigated. Therefore, cytogenetic results should preferably not be on-line to other areas of laboratory or hospital filing systems. If there is a networked computerised system, a special password security system should be in place. Filing of records should incorporate a security system to avoid access by unauthorised persons. Laboratory databases that contain patient information or test

results must be secure, password locked and backed up at regular intervals.

Appropriate measures should be in place to prevent unauthorised physical or electronic access, especially if the databases are located in non-secure premises, or are stored on networked computers.

Confidentiality agreements are to be signed by all members of staff with access to confidential patient information (Freedom of Information, 2000 and Data Protection Act, 1998).

For the transmission of facsimile results an appropriately worded cover page noting the confidentiality of the attached materials and instructions on what to do in case of accidental transmission to an inappropriate recipient should be included. Faxes should be transmitted to a secure fax. If there is no secure fax, the recipient should be notified before sending and acknowledge the receipt of the fax.

17. DOCUMENT CONTROL OF PROCEDURES AND PROTOCOLS

All protocols and methods used should be comprehensively documented and authorised by the director or supervisor of the laboratory section. Changes in protocols and methods should be dated so that for every procedure it is possible to deduce which protocol was used on a given day. All SOPs should have unique identifiers, a review date or date of issue, revision version, total number of pages and name of authoriser.

Annual re-evaluation of protocols, procedures and manuals is recommended. All changes should be dated and signed by the person responsible for the internal quality assessment. Obsolete versions should be retained for at least 10 years.

There should be clear document control such that it is clear which SOP version is current and all previous SOPs are collected to prevent use of invalid or obsolete documents. It should be evident who had a copy of the current SOPs.

There should be procedures for the identification, collection, indexing, access, storage, maintenance and safe disposal of quality and technical records.

18. HEALTH AND SAFETY (H & S)

If not covered and regulated by National regulations or EU legislation the following should apply.

There should be a person(s) appointed who is responsible for Health and Safety. A laboratory safety committee should have the mandate to oversee safe working practices in order to minimise injuries and infections occurring to staff, patients and visitors. The laboratory safety committee should ensure that national and international standards are met and maintained and staff are aware of their responsibilities relating to H&S.

There should be health and safety procedure in place that includes:

1. Action in the event of a fire

2. Action in the event of a major spillage of a dangerous chemical or clinical material
3. Action in the event of an inoculation accident
4. Reporting and monitoring accidents and incidents
5. Control of substances hazardous to health/risk assessments
6. Decontamination of equipment
7. Chemical handling
8. Storage and disposal of waste
9. Specimen collection, handling, transportation, reception and referral to other laboratories

Laboratories should keep a register of all referral laboratories it uses and all samples referred to another laboratory.

See EU Directives in reference section.

19. EQUIPMENT, INFORMATION SYSTEMS AND MATERIALS

19.1 EQUIPMENT

There should be an inventory of all laboratory equipment with data of purchase, manufacturer, and serial numbers. There should be a record of any contracted maintenance as well as equipment breakdowns. There should be a procedure for the procurement and management of equipment. All equipment should be calibrated and have a risk assessment completed before use by staff.

19.2 INFORMATION SYSTEMS (IT)

All IT systems should have a back-up and procedures for storage, archive and retrieval. In addition the data should be have secure passwords and if required, procedures in place for the safe and secure disposal of data.

19.3 MATERIALS

There should be quality control of materials that includes verification of identity on receipt; risk assessments; safe disposal; inventory of lot numbers (to allow for vertical and horizontal audit trails); batch testing or calibration where appropriate. For more information see international standards (ISO 15189: 2003 or ISO 17025:2000).

20. LAB STAFF EDUCATION AND TRAINING

There should be an appointed person responsible for staff training and education within the department. Effective staffing is a prerequisite for providing a high quality service. This includes both appropriate training and qualified staff provision for performing the technical work, analysis and supervision. A level of staffing is required that enables the laboratory to report results without unnecessary delay.

The laboratory should have a training program with written protocols for each aspect of the laboratory work undertaken, including information and advice on health and safety. Each trainee should have a named tutor responsible for ensuring that training is given to the appropriate standard.

Each member of staff should have a written job description and contract.

The laboratory should have a register to include information on basic education, courses attended, etc. for each staff member.

Staff should be encouraged to gain appropriate professional qualifications.

It is the responsibility of the Head of the Department to ensure that the staff are able to participate in continuing educational programmes relevant to the diagnostic repertoire of the laboratory.

21. PRE-EXAMINATION PROCESS **SPECIMEN RECEIPT**

There should be information for users that includes location, contact details, opening times, in addition to details of the diagnostic service offered and guidance on referral information and specimen bottles required. There should be procedures in place for specimen collection and handling. The laboratory should give each sample a unique identifier code to minimise cross-contamination or mislabelling when processing. If the referral card and specimen sample do not match, the laboratory should contact the referring clinician. If the clinician requests the sample still be set up, the referring clinician should put in writing that he/she will take responsibility for any error due to the mislabelling of the sample.

Written SOP's should be available for all diagnostic procedures. All procedures performed in the laboratory should be traceable (vertical audit trail). It should be possible to reconstruct who did what on a given day, which reagent batches were used, which protocols, etc.

22. EXAMINATION PROCESS **- ANALYSIS**

All analysis and examinations on the sample should be documented and traceable. Microscope verniers (coordinates) of cells analysed should be documented with the analysis sheets to enable relocation. Staff should not undertake analysis before they have been trained and authorised as competent. Competency may be determined by an 'analysis test'. All analysis should be validated by a second competent individual. For other aspects of the examination process please refer to the guidelines section.

23. POST-EXAMINATION PROCESS - **CHECKING AND AUTHORISATION**

A record of cultures and analysis should be signed by the responsible persons involved in the processing. Before any report leaves the laboratory it should be checked and signed by an authorised person. See guidelines section for more information on interpretation and reporting of results.

Stringent checking procedures should be in place in order to minimise errors in patient or sample identity.

The laboratory should have a documented system for checking the critical processing points of a sample. Storage or safe disposal of samples shall be according to local or national regulations.

24. INTERNAL AND EXTERNAL **QUALITY ASSURANCE (IQA & EQA)**

The laboratory should have a policy and procedure in place that can be implemented when it detects that any aspect of its' examination process (service) does not conform with its' own procedure. Procedures for corrective action should include an investigation process to determine the underlying cause(s) of the problem. If preventative action is required, action plans should be developed.

All operation procedures (managerial and technical) should be audited and reviewed by laboratory management at regular intervals.

24.1 INTERNAL QUALITY ASSESSMENT **(IQA)**

The internal quality control systems must verify the intended quality of results where this is quantifiable. Setting, monitoring and maintaining laboratory standards (IQA) should be the duty of the supervisor or another appropriately qualified named person.

He/she should set:

- band resolution levels appropriate for each referral category ,
- criteria for assessing the banding level,
- procedures for improvement when these banding levels are not met.

The band resolution levels must not be of a lower standard than that decided by National Guidelines. The head of the laboratory/department should receive frequent and periodic information regarding current laboratory performance.

Laboratories should regularly audit sample success rates and overall preparation quality. Where standards fall below the agreed criteria it should be possible to investigate the underlying reasons and then instigate measures to rectify any deficiency.

It should be ensured that any steps taken to investigate and rectify problems encountered are documented. Any procedural, analytical or reporting errors should be checked regularly.

24.2 EXTERNAL QUALITY (EQA)

The laboratory should participate annually in recognised EQA programmes appropriate to its full repertoire of analyses. EQA programmes should be recognised/endorsed by the Cytogenetic profession or a National Genetic Society.

If no National Scheme exists, European EQA schemes that are open to other countries are given on www.eurogentest.org website.

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APPENDIX

A. INDICATIONS FOR CYTOGENETIC ANALYSIS

Whenever a clinician suspects a patient's condition/disease is due to a chromosomal abnormality, he/she should consider a cytogenetic analysis. Although these conditions are well known to most clinicians referring patient to a cytogenetic laboratory, this list of indications may be helpful to delineate the type of patients eligible, especially if these indications are used in conjunction with the ICD-10 nomenclature of diagnoses. These indications are given as a guideline to enable stakeholders to monitor the referral pattern and the expected workload of a cytogenetic laboratory.

CLINICAL INDICATORS FOR CYTOGENETIC PRENATAL DIAGNOSIS

(Amniotic fluid, chorionic villi, foetal blood)

- previous livebirth with a chromosome abnormality
- previous stillbirth with a potentially viable chromosome abnormality
- parental chromosome rearrangement, chromosome mosaicism or sex chromosome aneuploidy
- positive maternal serum screening result indicating an increased risk of a chromosomally abnormal foetus
- increased maternal age
- abnormal foetal ultrasound
- resolution of possible foetal mosaicism detected by prior prenatal study
- risk of chromosome instability syndrome

CLINICAL INDICATIONS FOR INVESTIGATION OF CONSTITUTIONAL KARYOTYPE

(Peripheral blood, bone marrow, fibroblasts)

Significant family history of:

- chromosome rearrangements
- mental retardation of possible chromosomal origin where it is not possible to study the affected individual
- a relative with a history of pregnancy losses, a malformed foetus or stillbirth of unknown etiology

Patient with:

- primary or secondary amenorrhoea or premature menopause

- sperm abnormalities - azoospermia or severe oligospermia
- clinically significant abnormal growth - short stature, excessive growth, microcephaly, macrocephaly
- ambiguous genitalia
- abnormal clinical phenotype or dysmorphism
- congenital abnormalities
- mental retardation or developmental delay
- suspected deletion/ microdeletion/ duplication syndrome
- X-linked recessive disorder in a female
- clinical features of a chromosome instability syndrome, including isolated haematologic findings
- monitoring after bone marrow transplantation

Couples with:

- chromosome abnormality or unusual variant detected at prenatal diagnosis
- recurrent pregnancy losses (3 or more); stillbirths, or neonatal deaths where it is not possible to study the affected conceptus
- child with a chromosome abnormality or unusual variant
- infertility of unknown etiology

CLINICAL INDICATIONS FOR FISH TESTING OF CONSTITUTIONAL SPECIMENS

Individual with:

- a clinical suspicion of a microdeletion syndrome for which established diagnostic testing is available
- increased risk for a microdeletion syndrome because of a positive family history
- clinical features that suggest mosaicism for a specific chromosomal syndrome
- a bone marrow transplant for follow-up, when the donor is of the opposite sex to the recipient
- a chromosomal abnormality suspected by standard cytogenetic analysis when FISH testing may prove to be useful in further clarification of the abnormality or in situation where there is an important clinical implication
- presence of a supernumerary marker chromosome
- a clinical suspicion of a cryptic subtelomeric rearrangement, including relatives at increased risk for the cryptic subtelomeric rearrangement

Metaphase FISH

Evaluation of:

- marker chromosome
- unknown material attached to a chromosome
- rearranged chromosomes
- suspected gain or loss of a chromosome segment
- mosaicism

Interphase FISH:

Evaluation of:

- numerical abnormalities
- duplications
- deletions
- rearrangements

- sex chromosome constitution
- mosaicism
- gene amplification

Rapid Prenatal FISH/QF- PCR

- High risk of chromosome abnormality e.g. abnormal ultrasound

CLINICAL INDICATIONS FOR CANCER CYTOGENETICS

(bone marrow, lymph node, solid tumour, aspirates, fluids)

- Acute leukaemia: at diagnosis. If an abnormality is present, follow up after treatment or at relapse may be indicated. If an abnormal clone is not detected, re-investigation at relapse may be indicated.
- Myelodysplasia (MDS): at diagnosis, especially in the BMT-eligible patient. Follow up may be indicated at disease progression and after treatment.
- Chronic myelogenous leukaemia (CML): at diagnosis. Follow up may be indicated for staging purposes or to monitor therapy efficiency.
- Other chronic myeloproliferative disorders (MPD): at diagnosis in selected cases, to rule out CML and to assess for possible acute leukemic transformation.
- Malignant lymphoma and chronic lymphoproliferative disorders (CLPD): at diagnosis in selected cases.
- Solid tumours: may be indicated at diagnosis for small round cell tumours of childhood, selected sarcomas, lipomatous tumours, and other tumours in consultation with the pathologist/ clinician.

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D. INTERNATIONAL/EUROPEAN STANDARDS

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