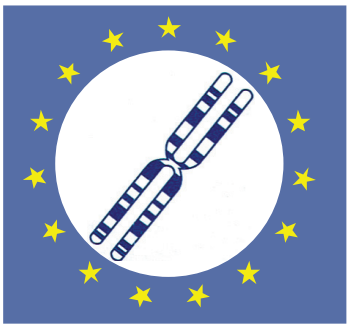
General Guidelines and Quality Assurance for Cytogenetics



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

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

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GENERAL GUIDELINES

Version 2.0

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, 'Eurogentest'.

These general 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. The general guidelines cover the generic aspects of diagnostic guidelines. For more specific guidance on constitutional cytogenetics, acquired cytogenetics, microarrays and report writing refer to the specific European guidelines in Appendix D.

These general guidelines take into account the existing quality assessment (EQA) schemes, good laboratory practice 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 participation in EQA and accreditation of cytogenetic laboratories. The OECD guidelines (2007) encourage genetic testing laboratories to participate in EQA (proficiency testing), establish a quality management system and become accredited.

The use of 'must' in this document indicates a requirement, when not in conflict with national law or regulations, and the use of 'should' or 'may' indicates a recommendation.

Some genetic tests could be performed with a variety of technologies and the laboratory needs to select the appropriate test given the sample type and referral reason. 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, microarray analysis or molecular genetic techniques may be more appropriate to detect the abnormality than routine chromosomal analysis. In addition, supplementary tests may be required to ascertain the results. Cytogenetic services must therefore keep up to date with advancing technology as it shifts from a cytogenetic to a more molecular genetic application. In view of rapidly changing practices and technology, the guidelines will be continually revised by the Permanent Working Group.

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 a referral 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 must 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 Molecular Cytogenetics
- Ph.D. with specialisation in Genetics and Cytogenetics or Molecular Cytogenetics
- Degree (e.g. B.Sc. or M.Sc.) with specialisation/experience in Genetics and Cytogenetics or Molecular Cytogenetics
- National registration with specialisation/experience in Genetics and Cytogenetics or Molecular 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 with specialisation/experience in Genetics and Cytogenetics or Molecular Cytogenetics
- National registration with specialisation/experience in Genetics and Cytogenetics or Molecular Cytogenetics

Troubleshooting in cytogenetics (constitutional, acquired molecular cytogenetics or molecular cytogenetics) 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 respond to general enquiries to the department.

2.7 Medical collaboration

The laboratory should have access to medical expertise on a 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.

For some referrals, for example acquired cytogenetics, a close and timely liaison with the referring clinician or other related pathology disciplines is required to clarify diagnosis, ensure appropriate culture, analysis, interpretation of findings and particularly in rationalising inappropriate (*e.g.* reactive) samples.

2.8 Scientific collaboration

The laboratory should encourage research and scientific collaboration. For instance, if a laboratory is validating a molecular technology (*e.g.* MLPA, QF-PCR), an appropriate molecular genetics 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. DIAGNOSTIC 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, laboratory organisation, 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 and cover is provided during absence or vacations.

Obviously the workload will vary depending on the complexity and weighting of the different tissues within the laboratory *e.g.* a reduced workload is appropriate in laboratories where a more complex or technically difficult oncology, FISH, or array-CGH specimen predominates.

The laboratory workloads adopted must guarantee maintenance of the ISO Standard. Sufficient time should be allocated to developmental work and continuous professional education (CPD/CME) of staff (see section 17 – Lab staff education and training).

Once a technique has been established, a laboratory should process a minimum of 100 samples per year in a given cytogenetic field (constitutional pre- and postnatal or acquired) to maintain the level of expertise. 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 the 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. In addition, there should be re-training or revalidation of staff after returning from any extended sick or maternity leave (>3 months).

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 and conform to Health & Safety as well as ISO15189 standards. For accredited laboratories the work environment must comply with ISO15189.

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

4.1.1 Referrals

See Appendix A for indications for referral to a cytogenetics laboratory. The laboratory should have policies for onward referral where cases require specialised expertise not provided locally *e.g.* chromosome breakage analysis, microarray, whole genome sequencing genetic testing.

When tests have been delegated to another laboratory, the originating laboratory should request and receive copies of the reports from that laboratory. The originating laboratory must retain responsibility for the interpretation of their own results in the context of the additional information obtained. Accredited laboratories should refer samples to another accredited laboratory (ISO 15189, 2007).

4.1.2 Consent

Informed consent must be given for all Cytogenetic and Molecular Cytogenetic tests. The patient must be made aware that other chromosome abnormalities may be detected with serum screening positive tests or maternal age referrals *etc.*

Cystic Fibrosis testing must NOT be done for QF-PCR rapid aneuploidy testing unless informed consent is given.

Due to the high frequency of CNV in the human genome, high-resolution arrays of DNA from a normal individual reveals an individual 'signature' of copy number variants. Thus, if high-resolution microarray/ arrayCGH of parental samples is used to determine whether copy number variants are *de novo* or inherited, the potential to reveal misattributed parentage, for example non-paternity, is possible. Homozygosity mapping using SNP arrays has been a powerful technology for identifying novel autosomal recessive genes in highly consanguineous families. When applied to 'molecular karyotyping' the potential for SNP arrays to reveal consanguinity should be kept in mind. These aspects should be considered by the clinician when obtaining consent for high-resolution molecular karyotype analysis.

4.1.3 Standard Operational Procedures (SOPs) and Validation

Standard operational procedures, for techniques or use of equipment, must exist for all operational procedures in the laboratory. SOPs must minimise the risk of sample mix-up. They must be written in a language understandable for the staff and updated annually. Obsolete versions of SOPs should be kept in an accessible format according to National regulations.

All new techniques must be validated prior to introduction into the diagnostic service. 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.

Any new batch of labelled probes, whether generated in-house or purchased commercially, requires validation concerning its performance before being used diagnostically. Any validation data must be fully documented for later internal audit. In house validation requires testing for target specificity as well as analytical sensitivity and specificity. Sensitivity and specificity must be high to avoid misdiagnosis.

- 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. However, it is still important that the first time a new probe, commercial or in-house, is used that a laboratory identifies the proportion of signal patterns in normal and abnormal samples before introducing the FISH test into a diagnostic setting.

4.1.4 Data analysis

The statistical methods used must be described in the laboratory's procedure manual. There must be internal criteria for deciding if the data produced is suitable for analysis. Analytical procedures and the checking systems used must be documented and specify the minimum level and experience of the staff involved.

Software packages should produce diagrammatic and numerical outputs for analysis. Software parameters must be set to ensure detection of imbalance at, or greater than, the level specified by the laboratory.

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.

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 any equipment failure affecting the laboratory work.

All equipment should be routinely maintained and records should be kept. All electrical equipment should be regularly tested for safety and all documenttation should be retained.

To avoid unnecessary delays due to equipment faults/failure, a service agreement is highly recommended for major items of equipment (*e.g.* image analysis systems).

4.2.1 Safety cabinets

All fresh biological 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). Many countries have national regulations for the protection of workers, samples and the environment. If no national regulations exist it is recommended to consult one of the following documents: - EC Directive (93/88/EEC), HSC, Advisory Committee on Dangerous Pathogens; the management and design and operation of microbial containment laboratories (ISBN9780717620340) 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 CO_2 (where used) controls. It is recommended that centrally monitored alarm systems are available.

4.2.3 FISH equipment

A dedicated work area should be available for FISH work. Specialised equipment should include facilities for incubation at varying temperatures, micro-centrifuge, fluorescence 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.

4.2.4 Array-CGH equipment

A dedicated work area should be available for array-CGH/SNP microarray work. Laboratory facilities must provide the appropriate working environment and equipment suitable for this technology. Hardware and software should be suitable for the microarray platform used and operate with appropriate levels of sensitivity and specificity to detect imbalances at or above the size cited by the diagnostic service.

4.2.5 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.

4.3 DNA SPECIMENS

Microarray, QF-PCR and MLPA analysis may be performed on any specimen that yields DNA (*e.g.* peripheral blood, cord blood, skin fibroblasts, fixed-cell pellet, and paraffin-embedded tissues). The laboratory must establish the specimen requirements for the technique for each tissue type and needs to determine the normal and abnormal threshold values for each sample type, recognizing that the sensitivity of the assay may differ for each.

4.3.1 DNA sample processing

The minimum DNA required to perform the molecular test (Array, QF-PCR, MLPA) for the specified platform should be established.

The laboratory must have written procedures (SOPs) for DNA extraction and labelling, DNA quantification, obtaining adequate quality and concentration of DNA, proper fragmentation, and if applicable adequate fluorescent labelling. The laboratory should be able to audit trail these parameters for each patient test.

All techniques employed must be subject to internal quality control. All manual sample transfers or loading of robotic systems must be checked by a second individual (if no bar-coding system or spiked DNA sample used) or alternatively validated by another method and suitably documented. All methods must ensure a minimal number of tube-tube transfers and produce a standard quality of DNA that is reliable for use in microarray assays. There must be internal criteria for deeming the DNA quantity or quality as unsuitable.

4.4 CYTOGENETIC ANALYSIS

4.4.1 Clinical indications for cytogenetic analysis

This may be dependant on local referral policy. However, it is not always appropriate to provide cytogenetic analysis for all referral categories. Appendix A gives some guidance on clinical indications where require cytogenetic analysis is appropriate.

4.4.2 Cell cultures

Duplicated or independently established cultures, where possible, are recommended for postnatal and haematology-oncology cultured specimens (see European Constitutional Guidelines and European Acquired Guidelines). At least two, preferably three cultures are recommended for prenatal cultured samples so appropriate work-up can be instigated if mosaicism is found (see European Constitutional Guidelines).

4.4.3 Suboptimal samples

If a sample does not meet requirements of the laboratory and is deemed suboptimal, the recommended action is to reject the specimen and request a repeat. If obtaining a repeat specimen is not possible, FISH, QF-PCR, MLPA or whole genome amplification could be considered if the laboratory is experienced in these techniques.

4.4.4 Banding

All karyotyping should be carried out using a banding technique. G-banding is the most widely used technique as it reliably gives the maximum level of band resolution (see Table 1). If other banding techniques (*e.g.* R- and Q-banding) are used the laboratory must ensure that the resolution is equivalent to that achieved by G-banding (550bphs). The vast majority of samples will require a full analysis of the banding pattern for the whole chromosome complement. In a few cases such as chromosome breakage syndromes, tumours and CLL other techniques such as solid staining or FISH will suffice.

ISCN defines five levels of banding. Several national guidelines have made recommendations for the degree of resolution required for a given referral indication and type of tissue (websites: ACC, www.cytogenetics.org.uk under info and BVDH, www.gfhev.de select 'Leitlinien/Stellungnahmen'). Germany and the UK also use an alternative approach that designates a quality score representing which chromosome bands are visible at the various bphs resolution *i.e.* 300 (QAS 3), 400 (QAS 4), 600 (QAS 6), 700 (QAS7), 900 (QAS9). A guide for assessing whether the banding quality (minimum) is acceptable for the reason for referral is given below in Table 1.

Full analysis must be completed to the satisfaction of the supervisor that numerical and structural abnormalities have been excluded to the minimum level appropriate for the referral reason (Table 1). Where it is not possible to achieve the minimum quality for the referral reason, and no abnormality is detected, the report should be suitably qualified whilst not encouraging a repeat invasive procedure when this is NOT clinically justified.

Table 1.

Reason for referral (Constitutional)	MINIMUM G-banding quality (QAS)
Confirmation of aneuploidy *e.g.* direct lymphocyte, direct CV or solid tissue culture preparation.	QAS 2 Ξ <300 bphs
Exclusion of known large structural rearrangements, e.g. lymphocyte, solid tissue, CVS direct preparation or amniotic fluid cell preparation	QAS 3 Ξ 300 bphs
Identification and exclusion of small expected structural rearrangements, e.g. lymphocyte, solid tissue, CVS culture or amniotic fluid preparation Routine amniotic fluid and CV culture preparations	QAS 4 Ξ 400 bphs
Other postnatal referrals (*e.g.* intellectual disability, birth defects, dysmorphic children or couples with recurrent pregnancy loss)	QAS 6 Ξ 550 bphs*
For microdeletion syndromes (when no FISH probe is available)	QAS 7 Ξ 700 bphs*

*arrayCGH/microarrays or other molecular techniques may be more applicable for some of these referral categories.
For acquired referrals, there is no minimum banding quality as this may be lower in the neoplastic cells compared to normal cells.
bphs = bands per haploid set

4.4.5 Chromosome analysis

The laboratory should have written protocols for the analysis criteria. Incomplete/broken cells should not be included in the analysis.

Metaphase analysis must involve a comparison of every set of homologues (including X & Y chromosomes), band by band. If one of the homologue pair is involved in an overlap with another chromosome the pair of homologues should be independently scored in another metaphase to ensure there is no structural rearrangement. Therefore, additional cells have to be counted and analysed to complete the analysis. The analysis may be undertaken by a trained technician.

For guidance on the minimum number of metaphases that should be analysed for each tissue type see Table 2 below. In general, a minimum of two cells must be fully analysed (at the minimum quality for the referral reason) for constitutional analysis, although in practice more metaphases are counted and analysed to clear any crossovers. An independent check of the analysis should be done by a second analyst (see Section 4.4.7). However, for acquired samples, the level of analysis depends on the disease, whether it is clonally abnormal or normal as well as whether it is a diagnostic sample or follow up sample (see the specific European Acquired Guidelines, 2011). Laboratories may choose to analyse or count more cells routinely than the minimum given in Table 2 to exclude mosaicism for all referrals - not just where suspected from the referral information.

Table 2

Tissue	Referral/Result	Minimum Analysed[a]	Additional cells counted
Postnatal Constitutional	Routine	2	0
Post- and Prenatal Constitutional	Mosaicism exclusion or single cell anomaly deletion	2	27
Prenatal Constitutional	Routine	2 (2 cultures)[b]	0 c
Post- and Prenatal	FISH metaphase	5	n/a
Post- and Prenatal	FISH interphase	100[d]	n/a
Prenatal screening	FISH interphase	30[e]	Up to 50
Acquired diagnostic	Normal	20	0
Acquired follow up	Abnormal	Variable	Up to 20[f]

[a] Number of metaphases fully analysed (comparison of every set of homologues)
[b] QF-PCR and 1 culture if aneuploidy.
[c] extra cells may be counted to exclude mosaicism or to exclude a single cell anomaly
[d] for mosaicism exclusion of a single cell anomaly (see Table 3).
[e] for each probe in anueploidy screening kit.
[f] screen for the previous clone.

An extended analysis and/or cell count is warranted when mosaicism is clinically indicated or suspected. Refer to the ISCN nomenclature for the definition of a clonal abnormality.

4.4.6 Image capture systems

When using image analysis systems, protocols should be in place to ensure that small markers or additional chromosomes from overspread metaphases have not been overlooked.

4.4.7 Checking

Checking of all cases by a second qualified cytogeneticist is essential. This independent check should involve a single comparison of every set of homologues as a minimum at the required quality for reason the referral reason). The same cells as the primary analyst may be used for the check. A senior supervisor or an experienced cytogeneticist must check the analysis. An independent 'blind' analysis where the checker does not know the first analyst's finding is recommended.

4.4.8 Interpretation

All cytogenetic reports must include an interpretation (see Section 6). The limitations of the test must be clearly given. Interpretation of results requires the supervision of a Section Head.

4.5 FLUORESCENCE IN-SITU HYBRIDISATION (FISH)

4.5.1 Analysis

Interphase and metaphase FISH, either as a single probe analysis, or using multiple chromosome probes, can give reliable results in different clinical situations (Table 3). 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.

Table 3

Probe type	Analysis	Additional comments
Locus-specific probes	5 metaphases	Score to confirm or exclude an abnormality.
Multiprobe analysis	3 metaphases	Per probe. Scored to confirm a normal signal pattern. Confirmation is advisable for abnormal signal patterns.
Prenatal interphase screening for aneuploidy	> 30 cells	For each probe set.
Interphase screening for mosaicism	> 100 cells	For each probe set.

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 (Table 3). Staff need appropriate training on the types of samples to be analysed. Laboratories should set standards for classification of observations and interpretation of results. When hybridisation is not optimal, the test should be repeated. The analyst should analyse sufficient numbers of cells depending on the probe type, see Table 3.

Most FISH results should be followed up by karyotype analysis. This is essential when there are discrepancies between the expected laboratory findings and the clinical referral.

4.5.2 Metaphase 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 performed 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.

4.5.3 Detection of single target probes

Commercially available probes/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 - usually five metaphases is adequate. If microduplication is suspected, results should preferably be confirmed by alternative methodologies (*e.g.* molecular analysis, densitometry).

4.5.4 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. Interphase analysis should involve the analysis of at least 100 nuclei.

It should be noted that interphase FISH analysis can 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 on cultured or uncultured cells may be an adjunct test to assess levels of mosaicism or

chimerism of cell lines with abnormalities previously established by standard chromosome analysis.

4.5.5 Checking

Interphase FISH results must be independently scored by an appropriately trained person. The checker should examine 30-70% of the total of cells used by the primary analyst. If the analyst and checkers' primary scores differ significantly then a third person (if necessary from another laboratory) must be called in to provide a resolution. This person should normally be informed of the previous scores.

For metaphase FISH the same procedures must be used as for checking conventional chromosome analysis.

4.5.6 Interpretation of results

The limitations of the 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 physician or cytogeneticist.

4.6 MICROARRAY

4.6.1 General

Microarray analysis looks at the whole genome at a specified high resolution. Oligo and BAC array CGH combines both conventional CGH methods and the use of microarray platforms. SNP array additionally enables the analysis of the parental origin of the SNPs.

This technique cannot detect balanced rearrangements, some ploidy changes, some low-level mosaicism and mutations (nucleotide base pair changes). Uniparental disomy cannot be detected by CGH-based arrays but may be detected using SNP-based arrays. Microarrays are usually used for patients with multiple congenital abnormalities. It may be used prenatally but patients should be counselled that the results may be uninformative or uncertain.

An array may be used either as an adjunct to more established testing methods, such as routine chromosome analysis and targeted FISH assays, or as a primary diagnostic tool for detecting chromosomal abnormalities. Before introducing microarrays as a diagnostic tool, the technique must be validated. Staff also need appropriate training with regard to technical aspects, bioinformatics and data interpretation. Laboratories should set standards for classification of observations and interpretation of results. Written standards describing when and how the whole genome amplification procedure is performed should be incorporated into the laboratory manual.

For validation of a new lot of the same microarray established in the laboratory one (preferably) abnormal specimen is repeated on the new lot and compared with the result from the old lot to establish equivalency. The manufacturer should supply documentation of the quality control on the new lot.

4.6.2 Microarray resolution

Both BAC, oligo-based and SNPs arrays are successfully used in a clinical diagnostic setting. The resolution of molecular karyotyping is in the first instance dependent on the platform used.

The laboratory should establish the number of clones/probes used to determine an abnormal threshold. The real resolution should be given in the report. It is important that the patient has to be informed that the test might yield results unrelated to the clinical question but nevertheless of the importance for the individual health or health of the individual.

4.7 QF-PCR

See Constitutional Guidelines, Section 1.6

4.8 CONFIRMATION OF ABNORMAL OR AMBIGUOUS RESULTS

The laboratory should have a written protocol in place that allows for confirmation of abnormal or ambiguous results. This may include cytogenetic analysis using banding techniques, FISH, PCR, or microarray depending on the result and the original technique used. Interaction with the referring clinician and/or clinical geneticist is essential when an unusual cytogenetic finding is found.

Whether or not alternative techniques are chosen to confirm a specific imbalance depends on the degree of uncertainty for cytogenetic abnormalities (or for microarrays the probability that a perceived imbalance is a true positive 'call'). If this probability is low, small imbalances may be verified by independent molecular techniques including karyotyping, FISH, Q-PCR, MLPA, array studies or higher resolution arrays. For deletions, all these techniques will be accurate. For small duplications, FISH should be quantitative, since the resolution of fluorescence microscopes may not separate the signals derived from a duplication. The laboratory must have a written policy in place that deals with discrepant results, *i.e*., when an abnormality cannot be confirmed by other methods.

5. SUCCESS RATES

Success rates depend on sample quality on receipt and individual laboratory policies on processing substandard samples and the technique used to analyse the sample. 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 on the quality of results so that corrective action can be taken. These success figures are for samples received of adequate quality and should be achieved annually (Table 4).

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

Table 4

Tissue	Minimum success rate
Amniotic fluid and long term CVS cultures	98%
Direct CVS	90%
Postnatal peripheral blood samples	98%
Fetal Blood samples	98%
Haematology samples	95%
Solid Tumours	n/a*
Products of conception/Fetal parts/skin biopsy	60%**

*For solid tumour samples it is not possible to set minimum standards due to the diversity of samples.
**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.
If MLPA/FISH/QF-PCR is used to exclude the common trisomies the success rate will be higher, with the proviso that fetal tissue is examined.

6. REPORTING

6.1 GENERAL

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 (see OECD guidelines, 2007). Long reports should be avoided as this detracts from the clarity of the results.

Reports must be issued in a standardised manner, clear to read for the non-specialist, so it can be clearly understood by the recipient/clinician. The report will be inserted into the patient's notes and may be seen, not only by the referring clinician, but also by healthcare workers. When writing a report it is important to remember that it may also be made available to the patient. Authorisation of reports must be carried out by a Clinical Scientist.

Handwritten alterations must never be made to the report. Accreditation standards insist that validation procedures are in place to ensure no alteration of reports can be made after issue.

Proband samples may be referred singly or with parental blood samples, depending on local referral policy. A preliminary report may be issued on detection of an imbalance in the proband. Comment on the clinical significance may be made in the preliminary report if a phenotypic association is supported in the published literature, otherwise it is appropriate to report as having unknown significance e.g. a del(4)(p16.3p16.3) deletion involving WHS1 gene, in the absence of further information.

A final report of an imbalance must be issued after completion of follow-up studies on both proband and parental samples. A result with no significant imbalance (as defined by the laboratory criteria) may be issued without parental studies.

It is acknowledged that some reports will be complex and may only be fully understandable to referring Clinical Geneticists. It is not necessary to include details of practical processing, unless relevant. Any report should inform the clinician of the limitations of the techniques employed.

6.1.1 Substandard analysis

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

6.2 CONSTITUTIONAL STANDARD REPORT CONTENT

Laboratory records must be auditable so that the individual cells, slide or array analysed can be traced back through to the reagents used and receipt of sample. Internal analysis sheets must include the resolution levels of the banding techniques used and details of any additional banding techniques used. It is not necessary to include details of culture procedures, unless relevant, *e.g.* from direct or cultured CVS, direct or cultured tumour. Where FISH or another adjunct test *e.g.* MLPA is performed to confirm a cytogenetic finding but does not add any additional information it does not need to be included in the FISH or MLPA ISCN.

The report should include the following information, where applicable to the test:

- clinical indication of test *e.g.* chromosome analysis or FISH or microarray;
- date of referral and/or date of receipt and date of report;
- patient identification using two different identifiers, *i.e.,* full name and birth date;
- unique sample identifier;
- name of referring clinician;
- laboratory identification;
- reason for referral;
- tissue examined;
- total number of cells counted and analysed for mosaic constitutional results, all haematological disorders and interphase FISH (included in ISCN too);
- FISH manufacturer and probe if appropriate (may be given in the footer);
- 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 a normal microarray should in addition contain:

- description of array (manufacturer, array version);
- the real array resolution;
- limitations of the test used;
- identification of genome build used;
- summary statement, if no clinically significant imbalance was detected.

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;
- schematic representation of aberrant regions for illustration (optional);
- whether the cytogenetic result is consistent with the clinical findings, and/or an indication of the expected phenotype;
- assessment of recurrence;
- prenatal diagnosis in a future pregnancy, if applicable;
- 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;
- where appropriate request for follow up of family members at risk of the abnormality, starting with closest available relatives;
- onward referral for genetic counselling, if the referral has not been initiated by a Clinical Geneticist.

The report of an ABNORMAL microarray report should in addition contain:

- summary statement and/or karyotype designation using latest ISCN nomenclature if genomic imbalances are detected;
- the location of genomic imbalances (reporting also name and position of the first and last significantly aberrant probes and ideally those of the first and last flanking normal probes);
- the size;
- the gene content of the genomic imbalance including the name of any known syndrome(s) in the region. 'Gene content' may refer to specific genes that are clinically relevant. Where there are few genes involved, listing each is not prohibitive, or alternatively a quantitative statement can be given such as 'there are many genes in this region' or 'there are no genes in this region';
- reference to other investigations to clarify significance;
- identification of methods used in follow-up studies;
- a clear written description of genomic imbalances;
- clinical interpretation (whether consistent with the referral reason).

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

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 present. FISH ISCN or a FISH summary should be given when it adds information to the metaphase karyotype.

For FISH analysis 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 (exception -haematological/tumour FISH analysis where no metaphases). This is of extreme importance with abnormal prenatal FISH results, where irreversible clinical actions could follow.

See also analysis sections in the Constitutional or Acquired specific Guidelines for other details on reporting.

6.2.1 Polymorphic variants

Polymorphisms such as heterochromatin size, satellite size, fluorescence 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.

CNVs should not be reported but a record kept as they may be needed for future review.

6.2.2 Mosaicism and pseudomosaicism

In general, reports should not mention mosaicism or pseudomosaicism, if it is shown to be apparently non-clonal or is likely to be a cultural artefact after appropriate work-up (see Gardner and Sutherland, 2004).

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, 1997 or ACC collaborative study 1994).

6.2.3 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. If the XX/XY mosaicism greater than 10% in more

than one culture or is level III, further investigations should be done (*e.g.* QF-PCR) before reporting.

6.2.4 Commercial FISH probes
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 probe name and source should be given for each case and any limitations of the probe should be clearly stated in the report.

7. 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 results due to insufficient staffing or administrative procedures. The report should be sent out after completion of all the analysis and test results.
The laboratory should have a written policy for reporting time. Recommended maximum report times for 90% of the referrals are given below in Table 5:

Table 5

Amniotic fluid and long term CVS cultures	17 days
Lymphocytes cultures	28 days
Bone marrows and solid tumour cultures	21 days
Solid tissue culture	28 days
Short term CVS cultures (directs)	7 days
Urgent* lymphocyte, cord blood cultures	7 days
Urgent* bone marrows cultures (diagnostic samples)	7 days
Prenatal aneuploidy FISH screening/QF-PCR	4 days

For array referrals:

Prenatal referrals	17 days
Proband (no follow-up required)	60 days
Proband and parental samples referred together for arrays	90 days
Parental samples requested after the proband's array result	60 days from receipt of parental blood samples

*Urgent - those referrals where the result will have immediate implications for patient management.

These report times include 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 10 days.
A laboratory must have a policy for identifying urgent results samples. It is recognised that it is not possible to evaluate the true clinical significance of an imbalance detected in the proband without parental studies and there is variation in the time taken to obtain parental blood samples.

7.1 PRELIMINARY RESULTS

In most cases the issue of a preliminary report should be discouraged. In general preliminary results should be communicated verbally by a supervisor or qualified cytogeneticist, to the clinician with **a clear indication that the analysis is provisional** and include a comment on which types of abnormalities have not yet been excluded. When preliminary results are given, a verified hardcopy must be issued stating that a final interpretation will be issued later. Also the verbal communication must be documented on the patient's laboratory record of the information given, to whom, by whom and the time and date.

8. CLINICAL RECORDS and STORAGE

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

8.1 Records

8.1.1 Retention of documentation
Some genetic tests are only done once during a patient's lifetime and may have implications for other family members, so clinicians should be able to access to the original results many years later.

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

For array-CGH analysis the experimental conditions of the array experiments as well as image analysis files (i.e. GPR files) and data interpretation files need to be stored digitally in the laboratory and/or in the patient records.

For QF-PCR the data files need to be stored digitally in the laboratory and/or in the patient records.

Digital images should preferably be duplicated and stored separately for long term storage according to national law or regulations.

8.2 Specimen storage

If possible cultures or fixed cell suspension should be kept until the final report is written. Relevant informed consent should be obtained for long term storage if required by National law.

Relevant information to trace the processing of the case should be saved for at least 10 years, but preferably indefinitely, especially if abnormal. 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.

Prenatal cell cultures with unique rearrangements should, if possible, be stored at least until 6 months 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 for at least 2 years to allow reanalysis later in the disease process. Some laboratories keep supernatants indefinitely for research purposes.

Cytogenetic slides/images must be stored so as to include sufficient banded material for reassessment if required. A minimum of 2 analysed banded metaphases should be stored, either as slides, photographic images or as a digital image. Slides must be stored for 5 years if no computerised image files or photo negatives are kept, for 2 years if images are kept. Image must be stored with maintained accessibility according to national law or regulations.

FISH results that cannot be visualised using conventional chromosome analysis must be kept. A minimum of one informative cell from an image or slide for either interphase or metaphase FISH analysis must be kept for the period specified by national law or regulations (unless the information is transcribed into permanently accessible report formats authorised by senior clinical laboratory staff).

Results, including computerised images or photo negatives, must be stored according to national law or regulations, if possible indefinitely.

9. QUALITY ASSURANCE

9.1. GENERAL

The Quality System of each cytogenetic laboratory should be consistent with as well as comply with current national and international standards (recommended: ISO 15189:2007; OECD guidelines 2007 or ISO 17025:2005).

10. ACCREDITATION and CERTIFICATION

Some of the issues covered by accreditation bodies are given below (See sections 11 to 21) for laboratories that are not yet accredited. More detailed information can be found in the international standards documents (ISO 15189:2007 or ISO 17025:2005).

10.1 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 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 ISO 9001:2008 have a major impact upon the structure and content of standards used for laboratory accreditation.

Accreditation is peer group assessment that a laboratory's performance across the required standards is acceptable (but 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 (*e.g.* BELAC, BMWA, COFRAC, DAKKS, SAS, UKAS).

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

10.2 Certification

Certification is not the same as accreditation.

Certification is based upon standards such as ISO 9001:2008 which delineate the 'requirements for quality management systems' and are applicable to any activity.

Certification only confirms that a laboratory adheres to the standards. It does not assess whether the laboratory's performance is acceptable.

However, certification of the counselling process may, where appropriate, be used as a complement to laboratory accreditation.

11. 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.* CCKL, UKAS and others). The laboratory should have a quality policy which sets quality objectives and has a commitment to achieve continual quality improvement (1S0 15189; OECD, 2007). 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.

12. 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. The quality manual should cross reference to the ISO standards.

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).

13. DATA PROTECTION and CONFIDENTIALITY

Confidentiality of genetic information is of utmost importance. Genetic data may contain information that is of importance to individuals other than the person investigated. Therefore, cytogenetic results should preferably not be online 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. Confidentiality agreements are to be signed by all members of staff with access to confidential patient information (Freedom of Information, 2000).

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.

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 fax.

14. 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. 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. Obsolete versions should be retained for at least 10 years.

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

15. HEALTH and SAFETY (H & S)

If not covered and regulated by ISO standards, 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 event
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. If it is an accredited lab, it should send samples to another accredited laboratory.

16. EQUIPMENT, INFORMATION SYSTEMS and MATERIALS

16.1 Equipment

There should be an inventory of all laboratory equipment with date 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.

16.2 Information Systems (IT)

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

16.3 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 trials); batch testing or calibration where appropriate. All chemicals should have a risk assessment as to whether they are hazardous to health which includes disposal instructions and what to do if a spillage occurs or a person is splashed, inhales or digests the chemical. For more information see international standards (ISO 15189:2007 or 17025:2005).

17. 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.

It is the responsibility of the Head of the Department to ensure that trained staff are able to participate in continuing educational programmes relevant to the diagnostic repertoire of the laboratory. 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. Each member of staff should have a written job description and contract.

The Department Head should ensure that the staff responsible for reporting have enough competence to understand the clinical context of the testing so the report is properly formulated to the need for genetic counselling.

18. 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, it should be documented that the referring clinician was informed that he has to take responsibility for any error due to the mislabelling of the sample.

SOPs should be in place for specimen receipt.

19. EXAMINATION PROCESS - ANALYSIS

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.

All analysis and examinations on the sample should be documented and traceable. 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.

20. 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.

21. INTERNAL and EXTERNAL QUALITY ASSURANCE (IQA & EQA)

The laboratory should have a policy and procedure in place that can be implemented when it detects 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.

21.1 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 for example (list below is not comprehensive):

- band resolution levels appropriate for each referral category;
- criteria for assessing the banding level;
- minimum hybridisation efficiency, probe specificity and sensitivity values;
- minimum quality DNA parameters;
- minimum software parameters to detect an abnormality;
- procedures for improvement when minimum levels are not met;
- success rates.

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 procedureal, analytical or reporting errors should be checked regularly.

21.2 External Quality Assessment (National, European, CEQA)

It is recommended that laboratories participate in National and/or European/International EQA programs for all aspects of the diagnostic service annually. EQA programmes should be recognised/endorsed by the Cytogenetic profession or a national genetics society.

If no national scheme exists, European EQA schemes that are open to other countries are given on www.eurogentest.org website or http://www.ceqa-cyto.eu/cyton/Home .

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APPENDIX

A. INDICATIONS FOR CYTOGENETIC ANALYSIS

Whenever a clinician suspects a patients' 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 patients to a cytogenetics 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 cytogenetics laboratory.

CLINICAL INDICATIONS FOR CYTOGENETIC PRENATAL DIAGNOSIS (Amniotic fluid, chorionic villi, fetal 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 fetus;
- increased maternal age;
- abnormal fetal ultrasound;
- resolution of possible fetal 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.

Patient with:
- primary or secondary amenorrhea 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;
- multiple congenital abnormalities;
- mental retardation or developmental delay;
- suspected deletion/ microdeletion/ duplication syndrome;
- increased risk for a microdeletion syndrome due to a positive family history;
- X-linked recessive disorder in a female;
- clinical features of a chromosome instability syndrome, including isolated haematologic findings;
- monitoring after bone marrow transplantation;
- a malformed fetus or stillbirth of unknown etiology;
- third and subsequent consecutive miscarriage(s) or products of conception from the fetus.

Couple with:
- chromosome abnormality or unusual variant detected at prenatal diagnosis;
- unbalanced chromosome abnormality in the products of conception;
- 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 or known aberration detected before transplantation;
- a chromosomal abnormality suspected by standard cytogenetic analysis when FISH testing may prove to be useful in further clarification of the abnormality or in situations 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/MLPA
- High risk of chromosome abnormality e.g. abnormal ultrasound.
- Late gestation referral.

CLINICAL INDICATIONS FOR INVESTIGATION OF ARRAY-CGH
(Peripheral blood, fibroblasts, amniotic fluid)

Patient with:
- clinically significant abnormal growth - short stature, excessive growth, microcephaly, macrocephaly;
- abnormal clinical phenotype or dysmorphism;
- multiple congenital abnormalities;
- mental retardation or developmental delay;
- suspected deletion / microdeletion / duplication syndrome;
- X-linked recessive disorder in a female.

Prenatal
- Two or more pathological ultrasound anomalies (includes IUGR);
- Parent carriers of a chromosomal rearrangement;
- Previous chromosomally abnormal child;
- Delineation of a chromosome abnormality detected prenatally.

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 neoplasms (MPN): at diagnosis in selected cases, to rule out CML and to assess for possible acute leukemic transformation;
- Malignant lymphoma and lymphoproliferative disorders (LPD): 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;
- CLL (FISH only) for prognostic indications.

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