Association for Clinical Cytogenetics

PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

SOLID TISSUE BEST PRACTICE GUIDELINES (2010) v1.00

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CONTENTS

1 INTRODUCTION

These guidelines should be used in conjunction with all other Association for Clinical Cytogenetics Best Practice Guidelines.

Professional guidelines for cytogenetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA) [1] and by statute (Clinical Governance) while taking into account current practice in the U.K.

The Human Tissue Act (2004) [2] established the Human Tissue Authority to regulate activities concerning the removal, storage, use and disposal of human tissue. It covers England, Wales and Northern Ireland. There is separate legislation in Scotland – the Human Tissue (Scotland) Act 2006 [3]. All establishments handling human material are required to have a licence.

The HT Act covers material, other then gametes, which consists of or includes human cells. It includes residual material following clinical and diagnostic procedures. It does not include cultured cells. A definitive list is available [4,5].

Elements of the service not subject to statute may be varied in order to comply with local constraints and agreements. These guidelines are minimum requirements and professional judgement is of paramount importance for many circumstances.

The use of 'must' in this document indicates a requirement and the use of 'should' indicates a recommendation.

Where there appears to be contradiction between available guidelines, the most recently published should be taken to apply to all.

All diagnostic cytogenetics laboratories must be accredited to nationally or internationally accepted standards. Laboratories must participate in an external quality assessment scheme for all aspects of their service for which a scheme is available [6].

It is recognised that there is no longer a consensus as to what technology should be used to determine whether a tissue sample (e.g. from a fetus or fetally-derived material) is euploid with or without balanced rearrangements or aneuploid (involving partial or complete gain or loss of whole chromosomes). These guidelines refer principally to classical karyotyping involving cell culture. Laboratories which use alternative methods of diagnosis must ensure that they are compliant with ACC best practice guidelines for such tests.

2 CONSENT

If consent for cytogenetic testing is unclear (e.g. a non-cytogenetic form has been sent or cytogenetics has not been listed as a required investigation), the relevant medical staff should be contacted to establish if testing is required and consent has been given. Any conversation should be documented.

3 TRANSPORT

Users of cytogenetic services should be encouraged to send specimens promptly as delay in reaching the cytogenetic laboratory is a common cause of culture failure.

Transport medium with antibiotics should be supplied for skin biopsies from live patients where feasible.

The transport of specimens by road or rail is regulated and requires P650 packaging. (See Health and Safety Executive website [7]). Cytogenetic laboratories must comply with the regulations. Users of cytogenetic services should be reminded of the regulations if they fail to act in accordance with them.

4 REASONS FOR REFERRAL

Referral reasons are a matter for local agreement and may include:

- spontaneous pregnancy losses of any gestational age, with or without congenital abnormalities
- TOP after abnormal ultrasound scan
- intrauterine death or stillbirth
- confirmation of prenatal diagnosis
- neonatal deaths
- sudden unexpected death in infancy (SUDI)
- investigation of mosaicism in dysmorphic/developmentally delayed individuals
- tissue specific syndromes
- feeder laboratory for tertiary referrals for testing other than cytogenetics, e.g. inborn errors of metabolism.

Local agreement may not include recurrent pregnancy losses; however, the Royal College of Obstetricians and Gynaecologists recommends that they are processed in cases where the mother is taking anti-coagulant therapy for anti-phospholipid syndrome[8]. This provides useful information for counselling and future management, e.g. if a normal karyotype is found, the anti-coagulant therapy can be altered.

The laboratory should have guidelines which can be issued to referring hospitals stating which referral categories are accepted and which tissues or samples are preferred.

The laboratory should have a policy for the rejection of excluded categories. This should include what is done with the samples and how the referring clinician is informed of the decision. If a sample is rejected, the clinician should be informed promptly and a written report issued.

5 CULTURE TECHNIQUES

5.1 General recommendations

It is recognised that maternal cell contamination can be a significant problem, particularly in placental tissue [9]. Care should therefore be taken to dissect off maternal decidua from placental tissue. It is strongly recommended that a dissecting microscope is used to select and clean chorionic villi or placental tissues, especially those from products of conception. Gentle scraping of the cord and membranes may remove adherent maternal cells.

The person responsible for selecting tissue for culture must be trained in the recognition of the various types of tissue encountered.

The laboratory must have a written policy for situations where there is no obvious fetal material.

At least two independent cultures should be set up if possible.

The risk of infection is higher for solid tissue referrals than for other cytogenetic referrals. Antibiotics should be added to the cell culture medium. Penicillin and streptomycin are effective antibiotics for routine use, which may be used in conjunction with an antifungal agent (e.g. amphotericin B, nystatin). Additional antibiotics can be used as required,

possibly under the direction of the Microbiology department if an infected supernatant has been sent to them for identification and sensitivity testing.

Harvesting all the cell cultures from any individual sample together should be avoided.

Whenever possible, a cell culture should be kept until the final report is issued.

5.2 Culture failure

In general, a culture can be regarded as having failed if there is no growth after 14 days. Before a case with an abnormal phenotype suggestive of a specific chromosome anomaly is 'failed', it is recommended that it is investigated by an appropriate molecular technique (e.g. FISH on interphase cells, QF-PCR or MLPA).

6 ANALYSIS

6.1 G-banded analysis

Refer to related Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) [10] for general guidance and for minimum banding score required for different referral reasons

Standard analysis

- Standard chromosome analysis for all sample types and cultures must be of a minimum of two metaphases and must consist of every pair of homologues being cleared in full at least twice at the minimum quality level appropriate for the referral reason.

- It is recognised that additional cells of varying quality may be examined in the analysis process without affecting the overall case quality score.

- Independent checking is an essential part of the analytical process.

- In cases with level III mosaicism, (two or more cells with the same chromosome abnormality distributed over two or more independent cultures) standard analysis must be carried out and one cell must be checked from each cell line.

- For *in situ* harvested preparations, each cell analysed should be from a separate colony.

6.2 Fluorescence *in situ* hybridisation (FISH)

Refer to related Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) [10].
Refer to related Professional Guidelines for Clinical Cytogenetics: Postnatal Best Practice Guidelines (2007) [11].

Metaphase FISH

When using site–specific probes on metaphases, 5 metaphases (suspension harvest), or 5 cells from more than 1 colony (*in situ* harvest) should be examined.
An independent check must consist of at least 2 cells.

Interphase FISH

	Number of nuclei examined		
	Analysis	Check	Total
Aneuploidy	20	10	30
Microduplication/ microdeletion	10	5	15

Depending on the quality of the preparations, higher numbers may be necessary to achieve a reliable result.

Checking should be carried out either on a raw image that has not been subject to enhancement, or by using a microscope. If using a microscope, a slide screening method should be employed to ensure that the analysis and check are performed on different nuclei. Alternatively, potential screening overlap can be circumvented by the 'total number of nuclei' being examined at both analysis and check.

7 ANALYSIS AND EXCLUSION OF MOSAICISM

Refer to related Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) [10].
Refer to related Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2009) [12].

More than one cell line may be present for a variety of reasons including:

- true constitutional mosaicism
- confined placental mosaicism
- unrecognized twin pregnancy or 'vanishing twin'
- chimaerism
- cultural artefact
- maternal cell contamination.

Mosaicism in one tissue or culture does not imply its presence or level in another tissue or culture, particularly one from a different embryonic lineage. Difficulties may be encountered if a normal cell line overgrows that of an abnormal line, or the abnormality (especially an extra marker chromosome) is lost in vitro.

7.1 Abnormal cell(s) found during routine analysis

Routine analytical protocols are not designed to confirm or exclude mosaicism, but when single or multiple abnormal cells are found during normal analysis extended scoring should be carried out.

When a single abnormal cell or multiple abnormal cells are found, the minimum work up should be a total of 30 cells scored from the same suspension culture as the abnormal cell, or 15 colonies from primary *in situ* cultures. Alternatively, the Hsu and Benn guidelines (1999) [13] (which involves looking at fewer cells but additional cultures) may be used as a basis for confirmation of true mosaicism (see Appendix 1, Tables 1 and 2).

Professional judgement should be used to decide if the additional work up is clinically justified. Any decision should be based on the type of chromosome abnormality, the reason for referral, the tissue that the abnormal cells have been found in, whether any additional information will help with interpretation of the result and the potential impact on recurrence. For example, extensive investigations of placental tissue will not give any information about the presence or absence of cells in the fetus itself.

7.2 Referrals for known mosaicism or suspected clinically significant mosaicism

Examples include:

- clinical details suggestive of known mosaic syndromes, e.g. trisomy 8, +i(12p)
- follow-up at birth of mosaicism of clinical significance detected by prenatal diagnosis (see also section 11 on confirmation of prenatal diagnosis)
- variation of skin pigmentation
- hemi-hypertrophy.

A minimum of 30 cells should be scored from a suspension culture or 30 colonies from primary *in situ* cultures. Subculturing should be kept to a minimum and preferably primary cultures used, especially with *in situ* culture techniques. FISH analysis may be the most suitable method of searching for mosaicism if suitable probes are available (minimum total of 50 cells for interphase FISH). FISH may also be performed on uncultured tissue.

8 ESACS (extra structurally abnormal chromosomes)

Refer to related Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2009) [12].

In fetal losses, the exhaustive identification of an ESAC is usually not clinically justified. A minimal work up and a request for parental bloods should be done concurrently as far as possible. The decision to proceed further will depend on the material still available, whether the ESAC is likely to have contributed to the pregnancy loss, whether it is familial (in which case studies can concentrate on the carrier parent) or, conversely, in consideration of the recurrence risk if it is found to be de novo.

In live patients, the origin should be established and further molecular or molecular cytogenetic techniques applied to ascertain the presence or absence of euchromatin. Euchromatin should be further characterized and UPD studies initiated if the marker involves chromosomes 7, 11, 14 or 15.

9 UNIPARENTAL DISOMY STUDIES

Refer to related Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2009) [12].
Refer to related Professional Guidelines for Clinical Cytogenetics: Postnatal Best Practice Guidelines (2007) [11].

Following detection of mosaicism in fetal losses, there is usually no need to carry out uniparental disomy (UPD) studies. They are rarely necessary since the outcome of them will not affect the recurrence risk, and potential cryptic mosaicism in the fetus could confuse the interpretation. Professional judgement should be used to decide which cases are suitable for UPD testing but should be restricted to those with a clinical phenotype associated with the chromosome and where the outcome will help with the interpretation of the result.

For live patients, UPD testing should be considered if the following is found:

- homologous or non-homologous Robertsonian translocations involving 14 and/or 15
- marker chromosomes of chromosome origin 7,11, 14, or 15
- apparent confined placental mosaicism for chromosomes 7, 11, 14 or 15.

10 MATERNAL CELL CONTAMINATION

Maternal cell contamination (MCC) is a particular problem when placental tissues are cultured [9].

Although there are other explanations for the existence of more than one cell line (see section 7 on mosaicism), maternal cell contamination should be suspected when:

- there is a mix of XX and XY cells (or mixed genotypes on QF-PCR)
- a female abnormal cell line is present together with karyotypically normal female cells
- the female karyotype is discordant with a previous prenatal diagnosis or fetal sex
- there is uncertainty about the identification of the tissue used in a culture
- there is slow cell growth, especially when originating from a single piece of tissue or a small number of colonies

If a female karyotype is discrepant to the stated fetal sex, firstly establish as far as possible that the correct fetal sex has been given. If possible, tissue from the fetus itself should be used to validate the result (e.g. by QF-PCR or FISH). Alternatively, cells can be scored from another culture, or QF-PCR or FISH can be used on uncultured tissue from the placenta. If appropriate, the presence of SRY should be tested for when a female karyotype is obtained, the fetus/infant is confirmed to be male and MCC has been excluded.

In cases where a female result is obtained from placental tissue and MCC is strongly suspected, then a suitable technique to validate the result should be employed (e.g. QF-PCR or FISH of the uncultured tissue or tissue from the fetus),

If maternal cell contamination is suspected, a rider should be added to the report to indicate the possibility of MCC (see Section 14).

11 CONFIRMATION OF CYTOGENETIC PRENATAL DIAGNOSIS

In general, laboratories should request/offer to confirm a chromosome abnormality seen at prenatal diagnosis or may be asked to do so in particular cases by referring clinicians. Aneuploidy/polyploidy may be confirmed by karyotype, molecular genetic or molecular cytogenetic techniques.

If a known mosaic pregnancy is lost or terminated, the amount of work up will be dependent on the tissues received, the abnormality involved and the level of mosaicism found at diagnosis.

If the known mosaic pregnancy is liveborn, the work up will be dependent on factors such as the presence of abnormal phenotypic features in the infant, whether or not the mosaicism is deemed to be possibly detrimental to the infant, or the presence of the abnormal cell line is to be investigated in a phenotypically normal infant. Cord blood may be a useful tissue in these instances. UPD studies may also be applicable (see section 9).

12 ALTERNATIVE INVESTIGATIONS

It is recognised that laboratories may elect, following appropriate validation and notification of change, to use one or more of these technologies instead of karyotyping for some of or all of the referrals they receive involving solid tissue samples.

12.1 FISH

Refer to related Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) [10].
Refer to related Professional Guidelines for Clinical Cytogenetics: Postnatal Best Practice Guidelines (2007) [11].

12.2 QF-PCR

Refer to related Professional Guidelines for Clinical Cytogenetics and Clinical Molecular Genetics: QF-PCR for the diagnosis of aneuploidy Best Practice Guidelines (2007) [14].

12.3 Array CGH

Refer to related Professional Guidelines for Clinical Cytogenetics: Constitutional Array CGH Best Practice Guidelines (2009) [15].

12.4 Other Molecular (Cyto)Genetics tests including MLPA

The laboratory should have a documented policy for dealing with referrals where molecular genetics testing is more appropriate.

13 REPORTING

Refer to related Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2009) [12].
Refer to related Professional Guidelines for Clinical Cytogenetics: Postnatal Best Practice Guidelines (2007) [11].

13.1 Reporting time

The reporting time refers to the issue of the final report, to include documented authorisation by an appropriately trained and qualified clinical scientist, and to be available on the departmental computer system in a form protected from revision.

95% of results of full karyotypes should be available within 28 days.

Reporting times should be auditable.

13.2 Report wording

See general Best Practice Guidelines (2007) [10] section 1.4 for full details of the expected content of reports.

The use of the word "abnormal" in describing a balanced familial rearrangement is discouraged.

13.3 Use of standard riders

Individual reports should include appropriate qualifying comments in the text when:

- it is not possible to achieve the minimum banding score for referral reason and no chromosome abnormality is detected
- there is considered to be a significant risk of an incorrect result due to maternal cell contamination. If it is suspected that the fetal karyotype has not been examined at all due to maternal cell contamination then parental bloods may be requested to exclude a structural rearrangement.

Note: The use of standard riders will not cover the lack of detection of an abnormality if an independent assessor determines that the abnormality should have been detected and, therefore, provides no legal protection.

13.4 Triploidy

If the parental origin of the extra haploid set has not been established then a statement should be included in the report to indicate that the triploid result may represent a partial hydatidiform mole. Partial moles are triploid in origin with two haploid sets of paternal chromosomes and one haploid set of maternal chromosomes. A diagnosis of partial hydatidiform mole has implications for the patient. [16]

13.5 Reporting mosaicism

Single cell abnormalities should not generally be mentioned in the report.

Where an abnormality is seen in multiple cells in a suspension culture, or in multiple cells in a single colony from an *in situ* culture (level II mosaicism) professional judgement should be used as to whether this is included in the report.

An abnormality seen in multiple cells in more than one suspension culture, or in multiple colonies from more than one *in situ* culture (level III mosaicism) must always be reported.

When mosaicism is reported, professional judgement must be used in the interpretation of the finding. This will depend on the type of chromosome abnormality, its level, the tissue(s) that the abnormal cells have been found in and the reason for referral. Caution must be applied in extrapolating findings from the placenta to the fetus. In many cases a definite conclusion will not be possible, in which case the various alternative explanations and interpretations should be covered.

The numbers of cells of each mosaic cell line must be included in the karyotype in square brackets [ ], as required by ISCN [17], and discussed in the report text.

13.6 Reporting of variants

- Well documented polymorphic variant chromosomes may not require reporting or family follow up [18]. Examples of variants that need not be mentioned in the report are detailed in Professional Guidelines for Clinical Cytogenetics: Postnatal Best Practice Guidelines (2007) [11]

13.7 FISH reports

- Reports of FISH analysis should be composed using the General Best Practice Guidelines (2007) [10].

14 STORAGE

Material must be stored in line with current good practice on security, traceability (records should detail the location of the materials) and health and safety.

All fetuses and fetal material must be stored separately in secure containers in a safe place prior to disposal. These containers should be made from opaque materials (in accordance with Department of Health guidance [19]) and be fit for transporting the tissue.

If material is stored for research, the requirements of the Human Tissue Act (2004) [2] and the resulting Code of Practice 1 [20] must be complied with.

15 DISPOSAL

The Human Tissue Act (2004) [2] and the resulting Code of Practice 5 [21] covers disposal of all human tissue but there are specific recommendations for the disposal of fetal material to take into account the particular sensitivities relating to it. Other documents also address this issue in some detail [22,23,24,25].

16 ARCHIVING OF SLIDES, PAPERWORK ETC.

This is covered in guidance issued by The Royal College of Pathologists and Institute of Biomedical Science: 'The retention and storage of pathological records and specimens', 4th edition (2009) [26].

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18 VERSION CONTROL

issue date	Current document	summary of changes	Version replaced
12/11/2010	**Solid Tissue Best Practice Guidelines 2010 v1.00**		No previous document

19 APPENDIX 1 - MOSAICISM

19.1 Table 1. Work up for analysis and exclusion of mosaicism – suspension culture

Suspension culture
Basic work up - a total of 20 cells from 2 independent cultures, one of which contains the anomalous metaphase. Single cell with: • 45,X • unbalanced structural rearrangement • balanced structural rearrangement • break at centromere with loss of one arm
Moderate work up - a total of 20 cells from one additional separate culture without the initial observation. • extra sex chromosome (SC MC) • trisomy involving chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SC MC) • 45,X (MC) • monosomy (other than 45,X) (MC) • marker chromosome (SC) • balanced structural rearrangement (MC)
Extensive work up - a total of 20 cells from each of two further separate cultures excluding the culture with the initial observation. • trisomy involving chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21, or 22 (SC MC) • unbalanced structural rearrangement (MC) • marker chromosome (MC)

SC single cell observation **MC** more than one cell observation

19.2 Table 2. Work up for analysis and exclusion of mosaicism – primary *in situ* culture

Primary *in situ* culture
Basic work up - a total of at least 15 colonies from 2 independent cultures. • all single cell abnormalities
Moderate work up - a further 12 colonies from further separate cultures should be examined. • extra sex chromosome (SC MC) • trisomy involving chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SC MC) • 45,X (MC) • monosomy (other than 45,X) (MC) • marker chromosome (SC) • balanced structural rearrangement (MC)
Extensive work up - 24 colonies from 2 further separate cultures. • trisomy involving chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21, or 22 (SC MC) • unbalanced structural rearrangement (MC) • marker chromosome (MC)

SC single colony observation **MC** more than one colony observation