PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

PRENATAL DIAGNOSIS BEST PRACTICE GUIDELINES: CHORIONIC VILLI (CVS) (2007) v1.01

March 2007
1 INTRODUCTION

These guidelines should be used in conjunction with the Professional Guidelines for Clinical Cytogenetics:
General Best Practice Guidelines (2007)
Prenatal Diagnosis Best Practice Guidelines (2005)
QF-PCR Best Practice Guidelines (2005)
For fetal blood samples, refer to Postnatal Best Practice Guidelines (2007)

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

The use of ‘shall’ 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.
2 TECHNIQUES

It is the responsibility of the laboratory to dissect the sample prior to set-up and to determine whether the material is suitable for processing.

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

2.1 Direct / short Term Cultures
The ACC Working Party (and other collaborative studies) have shown that best practice should involve the use of both direct/short term preparations and long term cultures [1]. In some laboratories, direct/short term culture has been replaced by rapid testing e.g. QF-PCR.

The techniques may involve either enzymatic or physical dissociation according to local SOPs.

Analysis from direct/short term preparations alone is not recommended but may be appropriate if there is limited material and the referral reason is for sexing prior to molecular test or a translocation carrier family. Analysis may be by chromosome analysis, FISH or QF-PCR.

2.2 Long Term Cultures

Three independently established cultures should be set up when possible; two are acceptable when a direct/short term preparation has been obtained.
3 REPORTING TIMES AND SUCCESS RATES

Full karyotype – long term culture:
95% of results of full karyotypes should be available within 14 calendar days.

Rapid Tests (by direct/short term cultures using chromosome analysis, FISH or QF-PCR):
95% results of rapid tests should be reported within 3 working days of receipt of the sample in the laboratory.

Long term Culture Failure:
This should be based on assessment of individual cases but in general: Laboratories should consider informing the clinic of a potential failure within 10 days, by telephone with the advice to contact the laboratory prior to repeating the sample. A final report should be issued for culture failure at 14 calendar days.

Success Rates:
Success rates are known to be affected by sample size and quality.

Laboratories shall strive to obtain a minimum success rate of
- 95% for analysis of metaphase preparations from freshly biopsied cultured material by direct/short term preparations (if 95% success rate is not achievable, FISH or QF-PCR studies may be more appropriate).
- 99% by FISH/QF-PCR on direct/short term preparations
- 99% for analysis of metaphase preparations from long term cultures
4 QUALITY FOR REFERRAL REASON

Refer to Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007)

4.1 Long Term Culture
Laboratories shall aim to attain the following minimum banding score for CVS samples.

The minimum banding score 4 for:
- Maternal age
- Biochemical screening
- Previous aneuploidy
- Anxiety
- Positive Nuchal Translucency screening test
- Soft markers which may be associated with but are not indicative of chromosome aneuploidy (e.g. choroid plexus cysts)

The minimum banding score 5 for:
- Fetal structural abnormalities visible on the ultrasound scan.

Where one parent carries a balanced chromosome rearrangement:
The banding score should be appropriate to detect/exclude the abnormality

Sub-optimal preparations:
Where it is not possible to achieve the minimum banding score for referral reason, and no abnormality is detected, the report should be qualified whilst not encouraging repeat invasive procedures when these are not clinically justified.

5 ADDITIONAL INVESTIGATIONS

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

Full chromosome analysis on samples referred primarily for a DNA or biochemical test (e.g. Cystic fibrosis) will be undertaken by local agreement.
6 ANALYSIS

Analysis of a Second culture is required:
When the result on a suspension culture is female and there is no consistent result from QF-PCR/FISH or chromosomes analysis result from direct/short term cultures

For abnormal results other than trisomy detected by QF-PCR/FISH or chromosome analysis on direct/short term culture.

It is acceptable to report on a single culture:
Where the result indicates that the fetal karyotype is different to that of the maternal karyotype, that is, when:

1. The result is male
2. The result confirms a trisomy found on QF-PCR/FISH chromosome analysis on a direct/short term culture
3. A parent carries a rearrangement and the female fetus:
   - has an unbalanced version of the rearrangement
   - has a rearrangement inherited from the father

For in situ techniques, analysis should include cells from more than one colony.

6.1 Chromosome analysis from Direct/short term cultures

Solid stain only
A minimum of 3 cells should be counted for a preliminary result. One cell must be independently checked.

G banded analysis
See standard analysis for long term cultures below.

6.2 Chromosome analysis from long term cultures

Standard analysis shall be of a minimum of two metaphases and shall 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. A minimum of one cell shall be analysed by the checker, with reference made to other cells when obscured regions of the karyotype need to be clarified, so that every pair of homologues is analysed at least once at the minimum quality level appropriate for the referral reason. In mosaic cases, one cell shall be checked from each cell line.

### 6.3 Analysis and exclusion of mosaicism

Karyotypic differences between cytotrophoblast, villus stroma, and fetuses are seen in 1 – 2% of CVS procedures investigated at 10 – 11 weeks [2]

Mosaicism from an early mitotic error can give rise to confined placental mosaicism (confined to the placenta or the foetus) or to generalised mosaicism (present in both the placenta and the fetus)

Mosaicism detected on laboratory analysis in cytotrophoblast (directs/short term) but not in stroma (long term) culture will usually be confined placental mosaicism. [3]

#### 6.3.1 For level I and II mosaicism:

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

In situations where a work-up cannot be fully achieved – clinical and scientific judgement should be applied. Further action depends on the chromosomes involved and professional judgment based on each case should be used.

A follow up amniocentesis is advised for the common trisomies and sex chromosome aneuploidy.

For situations where chromosomes are known to be involved in UPD, further studies should be considered. [4]

A detailed ultrasound scan should be advised.

#### 6.3.2 For level III mosaicism:

Amniocenteses or fetal blood sampling along with detailed ultrasound assessment of fetal morphology should be advised.
7 UNIPARENTAL DISOMY STUDIES

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

All laboratories shall have a clear written policy on the application of UPD studies in a prenatal setting. This policy should be produced in consultation with the appropriate Clinical Genetics/Molecular Genetics Department.

8 EXTRA STRUCTURALLY ABNORMAL CHROMOSOMES (ESACS)

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

9 FISH

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

10 REPORTING PROVISIONAL RESULTS

FISH - Refer to Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007)

It is acceptable to report provisional CVS results:

- for rapid detection of trisomy using FISH /QF-PCR
- fetal sex results from G banded analysis of direct /short term cultures
- fetal karyotype (eg 46,XX) results from G banded analysis of direct /short term cultures

The report shall include a statement indicating the limitations of the result and that analysis of cultured cells will follow.
The issue of a provisional result by rapid testing should have NO impact on reporting time guidelines for final report.

11 RETENTION, STORAGE AND DISPOSAL

Laboratories should comply with relevant guidelines regarding consent for testing, storage and disposal of material including:

- Royal College of Pathologists Retention and Storage of Pathological Records and Archives (3rd edition 2006)
- Statutory requirements of Human Tissue Authority See: Consent and Confidentiality in Medical Genetics Practice (April 2006) prepared by Joint Committee of Medical Genetics.

12 FOLLOW UP SAMPLES

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

In cases of possible CPM it is helpful to get placental material as well as fetal tissue for follow-up.

13 AUDIT OF PREGNANCY OUTCOMES

Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)
14 REPORTING

Use of riders
Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

14.1 Maternal Contamination

The possibility of maternal cell contamination should be included in the text of an individual report if there is considered to be a significant risk of an incorrect result.

Where maternal contamination is suspected consideration should be given to confirming fetal origin of cells using other techniques such as QF-PCR in liaison with the molecular genetics laboratory if appropriate.

For Females with one culture and no QF-PCR/FISH/direct/short term report: Refer to Professional Guidelines for Clinical Cytogenetics: Prenatal Diagnosis Best Practice Guidelines (2005)

When rearrangements are detected, specific risk figures need not be included, but where they are included the source of the information should be properly cited.

The use of the word “abnormal” in describing a balanced familial rearrangement is discouraged.
15 REFERENCES


2. Gardner RJM and Sutherland GR. Chromosome abnormalities and Genetic Counselling Third Edition 2004 Ch 25 p400-03


# 16 Version Control

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