Association for Clinical Cytogenetics

PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

PRENATAL DIAGNOSIS BEST PRACTICE GUIDELINES: AMNIOTIC FLUID (2005) v1.01

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CONTENTS

1 INTRODUCTION

These guidelines should be used in conjunction with the following documents;
Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007),
Prenatal Diagnosis Best Practice Guidelines: Chorionic Villi (2007),
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.

1.1 Prenatal Diagnosis

Prenatal diagnosis is offered to patients at risk of chromosome anomalies.

Reasons for referral will include the following:

- Abnormal ultrasound scan
- Carrier of a structural rearrangement
- Elevated risk indicated by biochemical and/or ultrasound maternal screening
- Increased maternal age
- Previous chromosome anomaly

Prenatal diagnosis is normally carried out using one of the following sample types:

- Amniotic fluid
- Chorionic villi
- Fetal blood

2 TECHNIQUES

Three independently established cultures should be set up when possible.

To minimise the risk of contamination or other problems in culturing, independent cultures should be handled separately, kept in separate incubators and maintained with different cell culture media or with different batches of the same basal media.

It is recommended that prenatal and non-prenatal cultures should be incubated separately to minimise the risk of microbial cross contamination.

Harvesting all the cell cultures from any individual sample together should be avoided. If possible a cell culture should be kept until the final report is issued.

3 REPORTING TIMES

3.1 Full Karyotype

Reporting times given refer to the issue of the final report, to include documented authorisation by an appropriately trained and qualified clinical scientist, and available on the departmental computer system in a form protected from revision

95% of results of full karyotypes should be available within 14 calendar days. (National Down's Screening Programme for England Annual Report 2004 [3])

Reporting times should be auditable.

Methods should be used to ensure that results are available at the clinic as soon as possible after authorising, e.g, by using secure electronic methods or secure fax.

There shall be compliance with legal requirements and constraints applicable to the communication of confidential information. (e.g. Health Service Circular HSC/1998/153 etc).

3.2 Rapid Tests

95% results of rapid trisomy screen (by FISH or QF-PCR) should be reported within 3 working days of receipt of sample in the laboratory. (National Down's Screening Programme for England Annual Report 2004 [3])

3.3 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

3.4 Success Rates

Laboratories shall strive to obtain a minimum culture success rate for amniotic fluid samples of 99%

4 ACCEPTABLE QUALITY FOR REFERRAL REASON

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

Laboratories shall aim to attain the following **minimum** banding score for AF samples.

The minimum banding score 4 for:

- Maternal age
- Biochemical screening
- Previous aneuploidy
- Anxiety
- Positive Nuchal Translucency screening test (10 - 14weeks)
- 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.

Based on current evidence, nuchal thickening seen in circumstances other than the first trimester screening test should be included in the abnormal USS category and should therefore be reported at banding score 5.

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

The consensus view is that it is acceptable to instigate additional tests where there are published associations without necessarily having explicit consent eg, TUPLE on a cardiac abnormality; whereas it would not be ethical to apply a test without clinical grounds eg, testing for CF mutations on mat age 35.

FISH for del(22)(q11) should be undertaken on prenatal referrals for heart defects, or outflow tract abnormalities only, according to local policy.

Extra counts/FISH should be done if reason for referral indicates risk of mosaicism e.g. ultrasound scan findings suggestive of Pallister Killian indicate i(12p) (see section 7).

For other additional investigations (not covered above) which are not requested on the referral card the referring unit should be informed that an additional test should be performed, the reason for doing so, and any anticipated delay in reporting – e.g. testing for breakage syndromes or UPD. These shall be carried out only after discussion and agreement with the referring Consultant.

For echogenic bowel referrals, testing for the common CF mutations should not be instigated without consulting the referring clinician and local molecular genetics laboratory.

Prenatal samples should not be tested prior to testing parental bloods for the appropriate common CF mutations.

Referral to Clinical Genetics should be considered if results on parental bloods are positive for one or more mutations.

6 NEED FOR SECOND CULTURES

Second cultures are required :
When the result on a suspension culture is female and there is no confirmatory QF-PCR/FISH result.

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.

7 ANALYSIS AND EXCLUSION OF MOSAICISM

Analysis of constitutional preparations comprises a minimum of three cells in which each banding region of each homologous pair has been cleared at least twice.

When single or multiple abnormal cells are found, Hsu and Benn guidelines (1999) [4] should be used as a basis for confirmation of true mosaicism.

7.1 For suspension cultures:

An indication for **basic work up** should lead to the examination of a total of 20 cells from two independent cultures, one of which contains the anomalous metaphase.

Where a **moderate work-up** is indicated, 20 cells from one additional separate culture without the initial observation should be examined.

An **extensive work-up** requires the examination of 20 cells from each of two further separate cultures excluding the culture with the initial observation.

7.2 For in situ cultures:

An indication for **basic work-up** should lead to the examination of a total of at least 15 colonies from two separate cultures

Where **moderate work-up** is indicated, a further 12 colonies from further separate cultures should be examined.

An **extensive work-up** requires the examination of 24 colonies from two further separate cultures.

See Table 1 below

Table 1

Suspension culture
a. Indications for **extensive work-up** • Autosomal trisomy involving a chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21 or 22 (SC MC) • Unbalanced structural rearrangement (MC) • Marker chromosome (MC)
b. Indications for **moderate work-up** • Extra sex chromosome (SC MC) • Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SM MC) • 45, X (MC) • Monosomy (other than 45,X) (MC) • Marker chromosome (SC) • Balanced structural rearrangement (MC)
c. Indications for **basic workup** Single cell with: • 45,X • Unbalanced structural rearrangement • Balanced structural rearrangement • Break at centromere with loss of one arm
In situ culture
a. Indications for **extensive work-up** • Autosomal trisomy involving a chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21 or 22 (SC MC) • Unbalanced structural rearrangement (MC) • Marker chromosome (MC)
b. Indications for **moderate work-up** • Extra sex chromosome (SC MC) • Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SM MC) • 45, X (SM MC) • Monosomy (other than 45,X) (SM MC) • Marker chromosome (SC) • Balanced structural rearrangement (MC)
c. Indications for **basic workup** • All single cell abnormalities
SC single cell observation MC more than one cell observation

Mosaicism considered to be an artefact by application of Hsu guidelines should generally not be mentioned in report. Particular care should be taken in interpreting level ll mosaicism (2 or more cells with the same abnormality in a suspension culture from a single flask, or in a single abnormal colony from an in situ culture) for a clinically significant aneuploidy. It may be appropriate to mention the finding in the report.

Interphase FISH may be used for the investigation of mosaicism in a prenatal setting (minimum count of 50 by FISH with application of reference range for specific probe being used).

It is not necessary to do extra counts (over the minimum noted above) on abnormal scan referrals, whether normal or abnormal result, unless there is a specific association with mosaicism.

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

8 UNIPARENTAL DISOMY STUDIES

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.

At present, adequate data is only available for CPM, additional marker chromosomes and balanced Robertsonian translocations. (Kotzot et al 2002) [5].

Systematic searches for UPD in balanced reciprocal translocations show no cases of UPD (although there are 5 cases in the literature, all of which involve chromosomes 15 and/or 16) and therefore UPD testing is not necessary in these situations.

UPD testing should be considered in cases of apparent CPM for chromosomes 7, 11, 14, and 15 and in homologous and non-homologous Robertsonian translocations involving 14 and 15, and marker chromosomes of chromosome origin 7, 11, 14 and 15.

UPD testing in mosaic trisomy 16 pregnancies (= a pregnancy with both disomy and trisomy 16 cells lines in the placenta and/or fetus). These cases present particular difficulties. In cases where UPD is excluded, there is still a significant risk of adverse fetal outcome (as judged by lower birth weight and/or fetal malformation. This is attributable to the presence of confined placental mosaicism or cryptic trisomy 16 mosaicism in the fetus or both. (Yong et al 2002 [6]; Yong et al 2003 [7]) For this reason it is particularly important that the implications of a negative UPD result are considered and understood before such testing is initiated.

9 ESACs (extra structurally abnormal chromosomes)

The following strategy is suggested for the identification of ESACs.

a) **Characterise as far as possible by routine cytogenetic methods**: G, C- banding or AgNOR staining if appropriate. Ensure spare slides/suspension are kept for FISH.

Points b) and c) below should be performed concurrently as far as is practical. Further testing is normally unnecessary once the origin of the marker is determined.

b) **Attempt to establish chromosomal origin by FISH**.

It is essential that chromosome 15 origin be excluded as soon as possible.

> All ESACs should have FISH with 15, 13/21 and 14/22 probes (whether satellites present or not).
>
> If the ESAC is not satellited then FISH with 15 α-sat,
> X, Y, if appropriate (i.e. chromosome count = 46)
> Probes for chromosomes conferring a risk of UPD i.e. 14,15
>
> In a prenatal setting the case for the exhaustive identification of an ESAC may not always be clinically justified. This particularly applies when it is shown to be familial or when a euchromatic, de novo ESAC is associated with significant fetal malformation detected on scan.
>
> Once the origin is identified, use of whole chromosome paint for that chromosome should be considered.
>
> Other probes may need to be used dependent on origin (e.g. mar 22 CES region, SNRPN)

c) **Request parental bloods in lithium heparin and EDTA as soon as possible to establish whether familial** (concurrent with ongoing FISH as above). Arrange for DNA to be extracted and banked for possible UPD investigation..

d) **Maintain remaining cultures for FISH and DNA extraction** (for possible UPD studies).

10 FISH

Refer to Postnatal Best Practice Guidelines (2007).

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 shall consist of at least 2 cells.

11 USE OF RAPID TESTS

Refer to Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007), QF-PCR Best Practice Guidelines (2005),

The issue of a provisional result by rapid testing should have NO impact on reporting time guidelines for final report.

12 FETAL BLOOD SAMPLES FOR PRENATAL TESTING

Refer to Postnatal Best Practice Guidelines (2007).

Fetal bloods should be verified as being of fetal origin by sender or laboratory.

Both amniotic fluid sample and fetal blood sample should be analysed unless there is a reason not to do so (e.g, abnormal FBS result and pregnancy terminated).

13 STORAGE

Laboratories shall 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 2005) [8]
- Statutory requirements of Human Tissue Authority

14 FOLLOW UP SAMPLES

a) Following an abnormal result and the pregnancy is terminated:

It is the responsibility of the referring clinic to decide whether confirmatory samples are sent on terminated pregnancies. If material is referred, confirmation of trisomy can be by FISH or QF-PCR if a full karyotype has been obtained

b) Following an abnormal result (balanced or unbalanced) and the pregnancy goes to term:

The laboratory should draw attention to the need to record the result in such a way as to facilitate counselling at the appropriate time. This may be achieved by recording this request in the prenatal report, requesting a blood sample at birth, or other local arrangement

15 AUDIT OF PREGNANCY OUTCOMES

Audit of pregnancy outcomes on an individual case basis is not required.

16 REPORTING

16.1 Use of standard riders

General limitations of prenatal diagnosis shall be included in information for Users, including:

- Subtle abnormalities – (see statement by ACC to the Royal College of Obstetricians and Gynaecologists dated 31/10/1994, available on ACC website)
- The risks of overgrowth of maternal cells in culture (MCC)
- The exclusion of mosaicism

Individual reports should include appropriate qualifying comments in routine text.

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.

Example riders/comments (not intended as an exhaustive list):

Normal or apparently normal

Normal karyotype – sex of fetus withheld at patient request

Please note that prenatal diagnosis may not necessarily exclude minor /subtle chromosome abnormalities or mosaicism

The preparations obtained from this sample are of sufficient quality to detect numerical and large structural abnormalities only

Routine prenatal chromosome analysis does not exclude the possibility of mosaicism and will not always detect small structural rearrangements

Banded analysis has been carried out to exclude the majority of numerical and structural chromosome abnormalities detectable with the light microscope

Analysis of cultured cells indicates a female karyotype with no gross chromosome abnormality detected.
This excludes numerical chromosome abnormalities (but not low frequency mosaicism) and larger structural rearrangements

This result should be recorded in such a way as to facilitate genetic counselling at an appropriate time.

16.2 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. Extra counts are not necessary.

For females with 1 culture and no QF-PCR/FISH report:

"*Only a single culture was available for analysis, therefore the risk of maternal cell contamination cannot be excluded, but the risk is considered to be low".*

Referral to Clinical Genetics is not necessary for common trisomies (i.e. 13, 18 & 21), but should be advised for all other abnormal results.

16.3 Report wording

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

17 REFERENCES

1. Standards for the Medical Laboratory. Version 1.03, (2004). Clinical Pathology Accreditation (UK) Ltd.

2. Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007); ACC website

3. National Screening Programme for Down's Syndrome. Annual report 2004

4. Hsu LYF and Benn PA (1999). Revised guidelines for the Diagnosis of Mosaicism in Amniocytes. Prenatal Diagnosis 19 1081-82

5. Kotzot D (2002) Review and meta-analysis of systematic searches for UPD other than UPD15 Am J Med Genet p366-375

6. Yong PJ, Marion SA et al (2002) Evidence for imprinting on chromosome 16; the effect of uniparental disomy on the outcome of mosaic trisomy 16 pregnancies Am J Med Genet 112 123-32

7. Yong PJ, Barrett IJ et al (2003) Clinical aspects, prenatal diagnosis and pathogenesis of trisomy 16 mosaicism J Med Genet 40 175-82

8. The Retention and Storage of Pathological Records and Archives. 3rd Edition. (2006), The Royal College of Pathologists, London.

18 Version Control

issue date	Current document	summary of changes	Version replaced
03/09/2007	**Prenatal Diagnosis Best Practice Guidelines: Amniotic Fluid 2005 v1.01**	updated referencing between ACC Best Practice Documents	Prenatal Diagnosis Best Practice Guidelines: Amniotic Fluid 2005
	Previous versions		
25/07/2007	**Prenatal Diagnosis Best Practice Guidelines: Amniotic Fluid 2005**	Note: Guidelines written 2005, reformatted 25/07/2007	

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