A. RECOMMENDATIONS FOR THE INDICATIONS, ANALYSIS AND REPORTING OF PRENATAL SPECIMENS

A.1 Indications for Prenatal Diagnosis

The Canadian guidelines for cytogenetic indications in prenatal diagnosis are fully described in the Clinical Practice Guidelines of the Society of Obstetricians and Gynaecologists of Canada (SOGC) (www.sogc.org/guidelines). Please refer to the following documents:

a) Prenatal Screening for Fetal Aneuploidy (2007) (document #187);
b) Fetal Soft Markers in Obstetric Ultrasound (2005) (document #162).

The Canadian guidelines for the techniques of prenatal diagnosis are also fully described in the Clinical Practice Guidelines of the Society of Obstetricians and Gynaecologists of Canada (SOGC) (www.sogc.org/guidelines). Please refer to the following document:

a) Amended Canadian Guideline for Prenatal Diagnosis (2005) Change to 2005 – Techniques for Prenatal Diagnosis (2005) (document #168).

A.2 Recommendations for Analysis of Amniotic Fluid Cultures

a) Flask method:

Count: minimum of 15 metaphases total, from two or more independent cultures.

Analyze: minimum of 5 metaphases from total, representing two or more independent cultures.

Karyotype: minimum of 2 cells from total, per cell line.

b) *In-situ* Method

Count: minimum of 10 metaphases total, each from a different colony, from two or more independent cultures.

Analyze: minimum of 5 metaphases from total, each from a different colony, representing two or more independent cultures.

Karyotype: minimum of 2 cells from total, per cell line.

The initial, routine work-up for most laboratories is 15-20 metaphase cells from at least two flasks or 10-16 colonies from at least two *in situ* culture vessels (Hsu et al, 1992). This work-up generally reflects the balance between the desired mosaicism detection sensitivity and laboratory costs (technical efficiency, costs, and turnaround time) (Cheng et al, 1995). The number of

colonies examined and the percentage mosaicism present will determine the ability to detect the mosaicism.

Analysis of amniotic fluid metaphases will not detect small structural anomalies and analyzing fewer cells than usual could further limit the detection of low level mosaicism and small structural anomalies.

References

Cheng *et al*. Is the 15-*in situ* clone protocol necessary to detect amniotic fluid mosaicism? Am J Obstet Gynecol173:1025-1030 (1995).

Hsu LYF *et al.* Proposed guidelines for diagnosis of chromosome mosaicism in amniocytes based on data derived from chromosome mosaicism and pseudomosaicism studies. *Prenat Diagn* 12:555-573 (1992).

A.3 Recommendations for Analysis of Chorionic Villus Cultures

CVS analysis should include an analysis of cultured mesenchyme. Additional testing by direct preparation or interphase FISH may be appropriate in some circumstances. Depending on the clinical correlation, follow-up with amniotic fluid or fetal blood culture may be helpful in investigation of mosaicism (refer to amniotic fluid guidelines for work up procedures and definitions of mosaicism).

To minimize the risk of maternal cell contamination, it is essential to clean villi from maternal decidua, blood vessels, membrane and other materials before tissue culture. A Cytogenetics laboratory should define a minimum amount of CVS sample needed for a routine and conclusive analysis. For a very small and/or bloody sample and finding of a normal female karyotype, a special caution should be taken in reporting such a result because of possibility of maternal cell contamination (MCC). Follow-up confirmation with amniocentesis or cordocentesis may be considered.

Count: minimum 20 metaphases from two or more independent cultures.

Analyze: minimum of 5 metaphases from total.

Karyotype: minimum of 2 cells per cell line.

Amniotic fluid represents a more diverse source of cells as compared to CVS, which represents a limited source of cells subject to confined placental mosaicism (CPM). Amniotic fluid would, therefore, better detect generalized mosaicism as compared to CVS, which may not detect generalized mosaicism because of its small sample size and/or location. Therefore, it is recommended that a minimum number of 15 metaphases be examined for the flask amniotic fluid method and a minimum at 20 metaphases be examined for CVS.

A.4 Recommendations for Analysis of Fetal Blood

As for constitutional karyotype (see below).

A.5 Recommendations for Evaluation and Interpretation of Mosaicism

These guidelines may be used by the cytogeneticist to interpret mosaicism in both amniotic fluid and CVS. Mosaicism in amniotic fluid can be evaluated by either the flask or *in situ* method, depending on the method of culture. Mosaicism in CVS can be evaluated by the flask method. However, the nature of the test is such that low level mosaicism will be missed with even the strictest of guidelines. **These are minimum guidelines. Each centre should establish their own policies for amniotic fluid and CVS mosaicism interpretation and reporting.**

a) Definitions

Worton and Stern (1984) originally described three levels of mosaicism based on the Canadian experience with amniocentesis from 1970-1980. The following definitions are consistent with Hsu *et al.,* (1992).

Level I (single cell pseudomosaicism, Hsu *et al.*, 1992): single cell anomaly.

Level II (multiple cell pseudomosaicism, Hsu *et al.*, 1992): same abnormality observed in two or more cells (flask method) or in two or more cells from one or more colonies (*in situ*) in the same culture (Winsor *et al.*, 1999).

Level III (true mosaicism, Hsu *et al.*, 1992): two or more cells with the same abnormality observed in two or more independent cultures.

b) Level I mosaicism

Work-up: No further workup is required for a single cell abnormality in an otherwise normal colony detected in a minimum analysis of 10 colonies from two or more independent cultures.

Reporting: Level I mosaicism should not be reported.

Follow-up: No follow-up or confirmation is necessary.

c) Level II mosaicism

Individual centres should establish local guidelines. Guidelines for the diagnosis of level II mosaicism are based on the three independent studies of Featherstone *et al.* (1994), Hsu *et al.* (1992), and Hsu and Benn (1999).

Work-up: Analysis of additional cells from other independent cultures is required, as summarized briefly below.

The most efficient and cost effective method to determine the clinical significance of level II mosaicism, according to Featherstone *et al*. (1994), is the analysis of additional metaphases such that the detection level of mosaicism approximates that of the original 10 *in situ* colony analysis. For example, to detect mosaicism of 27% or greater, with 95% confidence (Featherstone *et al.*, 1994) a minimum of 10 additional colonies from independent cultures should be analyzed for the same abnormality. To detect mosaicism of 26% or greater with a 95% confidence, a minimum of 20 additional colonies from independent cultures should be analyzed for the same anomaly. However, it is clearly recognized and stated by Featherstone *et al.* (1994) that the nature of the chromosome abnormality and its associated clinical outcome, as discussed by Hsu *et al.* (1992) and Hsu and Benn (1999), will influence the number of additional metaphases to be analyzed. For example, no additional work-up is recommended for single cells or colonies with a balanced structural rearrangement or break at a centromere with loss of one arm whereas an extensive work-up (i.e. an additional 24 colonies from independent cultures) is recommended for autosomal trisomy involving a chromosome 21, 18, 13, 8, or 9. Please refer to the table of guidelines for work-up in elucidation of possible amniotic pseudomosaicism/mosaicism made by Hsu and Benn (1999).

It is recommended, therefore, that the number of additional independent metaphases to be examined in addition to the original count be determined according to the type of cytogenetic abnormality detected (Hsu *et al.*, 1992, 1999) and the desired exclusion level of clinically significant mosaicism (Featherstone *et al.*, 1994). The specific number of additional metaphases should be determined by the unique nature of each case and is left to the discretion of the laboratory director.

In situations where the primary *in situ* culture dishes do not provide sufficient colonies for analysis and a flask culture needs to be used for analysis the probability table published by Sikkema-Raddatz *et al.* (1997) may be used to determine colony-equivalency for the flask culture.

Reporting: Level II mosaicism would usually not be reported. The cytogeneticist may elect to report “level II mosaicism” or “inconclusive result” under the following circumstances:

- analysis of a minimum number of additional colonies/metaphases is not possible [the number will depend on the abnormality identified (Hsu and Benn (1999)];

- if clinical findings, such as fetal anomalies, IUGR or suspected vanishing twin on ultrasound are present; and

- other circumstances (chromosomes known to be associated with clinically significant mosaic states) may also result in reporting of level II mosaicism.

Follow-up: For level II mosaicism that is reported, additional studies may be suggested at the discretion of the cytogeneticist.

d) Level III mosaicism

Work-up: No additional analysis is necessary.

Reporting: All level III mosaicism should be reported.

Follow-up: Additional studies such as fetal ultrasound may be suggested at the discretion of the cytogeneticist. Cytogenetic confirmation of the prenatal diagnosis should be performed in all cases of level III mosaicism. Analysis of more than one tissue may be required.

e) Maternal cell contamination (MCC) and diploidy/tetraploidy

Reporting of likely maternal cell contamination and diploidy/tetraploidy is left to the discretion of the cytogeneticist.

Cytogenetic laboratories should incorporate a mechanism for recording and retrieving data on mosaicism and outcomes, where possible.

References

Featherstone T *et al*. Exclusion of chromosomal mosaicism in amniotic fluid cultures: determination of number of colonies needed for accurate analysis. *Prenat Diagn* 14:1009-1017 (1999).

Hsu LYF *et al.* Proposed guidelines for diagnosis of chromosome mosaicism in amniocytes based on data derived from chromosome mosaicism and pseudomosaicism studies. *Prenat Diagn* 12:555-573 (1992).

Hsu LYF and Benn PA. Revised guidelines for the diagnosis of mosaicism in amniocytes. *Prenat Diagn* 19:1081-1090 (1999).

Sikkema-Raddatz B *et al*. Probability tables for exclusion of mosaicism in prenatal diagnosis. *Prenat Diagn* 17:115-118 (1997).

Winsor EJT *et al*. Cytogenetic aspects of the Canadian early and midtrimester amniocentesis trial (CEMAT). *Prenat Diagn* 19:620-627 (1999).

Worton RG *et al*. Canadian collaborative study of mosaicism in amniotic fluid cell cultures. *Prenat Diagn* 4:131-144 (1984).

A.6 Recommendations for Verification of Results of Prenatal Diagnosis

Experience from two large Canadian trials did not find any false positives for non-mosaic abnormal prenatal chromosomal results from amniocentesis samples (Lippman *et al.*, 1992, Winsor *et al.*, 1999). Therefore, cytogenetic confirmation of non-mosaic abnormal chromosomal results is not a requirement. For a discrepant phenotype/genotype outcome, cytogenetic confirmation should be attempted. For clinical situations where repeat testing after birth or pregnancy termination is considered, e.g. the possibility of misidentification of specimens etc, each center will establish its own policy.

In the case of mosaicism, cytogenetic confirmation should be attempted.

References

Lippman A *et al*. Canadian multicenter randomized clinical trial of chorion villus sampling and amniocentesis. Final report. *Prenat Diagn* 12:385-408 (1992).

Winsor EJT *et al*. Cytogenetic aspects of the Canadian early and midtrimester amniocentesis trial (CEMAT). *Prenat Diagn* 19:620-627 (1999).

A.7 Recommendations for Prenatal diagnosis and Parental Exposure to Radiation and/or Chemotherapy

The following statement is made in the Clinical Practice Guidelines of the Society of Obstetricians and Gynaecologists of Canada (SOGC) (www.sogc.org/guidelines). Please refer to document #105: Genetic Indications for Prenatal Diagnosis (2001).

Exposure to therapeutic radiation in males is associated with a significant increase in both numerical and structural chromosomal abnormalities in sperm, even years after treatment (Martin et al., 1986), however there is [sic] no data suggesting that the offspring of these men are at increased risk for aneuploidy or congenital malformation. As well, there is no evidence that eggs exposed to therapeutic radiation are similarly affected. Referral to a local genetics centre for evaluation is recommended.

While there are no data to suggest an increased risk for chromosomal abnormalities of offspring of individuals exposed to radiation and/or chemotherapeutic agents, individuals will vary with respect to their exposure. Furthermore, the decision to offer testing is ultimately a medical one

based on considerations that may include more than absolute risk and should be discussed by the referring physician on an individual-case basis with the testing laboratory and/or the prenatal program co-coordinator.

References

Martin *et al.* An increased frequency of human sperm chromosome abnormalities after radiotherapy. *Mut Res* 174:219-225 (1986).

A.8 Recommendations for Rapid Aneuploidy Detection (RAD) Test Set-Up & Reporting: Interphase FISH/QF-PCR for Prenatal Specimens

The American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories are suggested as an appropriate starting point for establishing and maintaining an interphase FISH (iFISH) prenatal diagnosis program. The Association for Clinical Cytogenetics and Clinical Molecular Genetics (British Society for Human Genetics) Best Practice Guidelines for the QF-PCR for the diagnosis of aneuploidy are suggested as an appropriate starting point for establishing and maintaining a QF-PCR prenatal diagnosis program. A laboratory that offers interphase FISH/QF-PCR testing on amniotic fluid or other prenatally obtained samples should, in addition to adhering to the above-mentioned standards, validate its results against known cytogenetic cases. The minimum number of samples to be analyzed per year for maintenance of proficiency is at the discretion of the individual laboratory. The laboratory must participate in an external proficiency program and meet that program's requirements.

Only unambiguous results should be reported in the absence of confirmatory cytogenetic information.

Whether the report should state that an irrevocable action should not be taken based on an amniotic fluid FISH/QF-PCR result alone, (e.g. prior to the confirmatory standard cytogenetic results) is a controversial issue. This issue may be mitigated if there are fetal ultrasound findings that are consistent with the amniotic fluid FISH/QF-PCR result. Each centre should establish its own policy regarding these cases. The issue of mosaicism in prenatal interphase FISH/QF-PCR has not been thoroughly evaluated in the literature. Abnormal results suggestive of mosaicism should be reported in conjunction with confirmatory cytogenetic analysis.

A.9 Recommendations for Average Turn-Around-Time for Completion of Prenatal Final Reports

The following reporting times are acceptable, keeping in mind that all laboratories are encouraged to reduce these reporting times whenever possible. At least 90% of all prenatal analyses should have final written reports within the recommended turn-around-time (TAT) listed below.

Amniotic fluid: 14 days

Chorionic villi: 21 days

Fetal blood: 7 days

Rapid Aneuploidy Detection (RAD) (iFISH/QF-PCR): 3 days

A.10 Recommendations for prenatal uniparental disomy (UPD) testing

UPD testing should be considered in fetuses at an increased risk of UPD syndromes as described in CCMG Statement on prenatal and postnatal diagnostic testing for UPD (pending for approval).

A.11 Recommendations for prenatal microarray testing

Microarray (array CGH and/or SNP array) may be considered for selected prenatal cases as recommended by CCMG Position statement: Use of array CGH technology in constitutional genetic diagnosis in Canada. Routine G-banding and/or FISH analysis may be needed for follow-up confirmation or providing additional information for some cases.

ABBREVIATIONS

CGH	comparative genomic hybridization
CPM	confined placental mosaicism
CVS	chorionic villus sample
FISH	fluorescence *in situ* hybridization
iFISH	interphase fluorescence *in situ* hybridization
IUGR	intrauterine growth retardation
MCC	maternal cell contamination
QF-PCR	quantitative fluorescence by polymerase chain reaction
RAD	rapid aneuploidy detection
SNP	single nucleotide polymorphism
SOGC	Society of Obstetricians and Gynaecologists of Canada
TAT	turn-around time
UPD	uniparental disomy