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CCMG Practice Guidelines for Cytogenetic Analysis

B. Recommendations for the indications, analysis and reporting of constitutional specimens (peripheral blood, solid tissues)

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B.1 Indications for Standard Cytogenetic Constitutional Investigations

This list includes some commonly accepted clinical indications for cytogenetic analysis. This list is not all-inclusive. Centers should set their own clinical indication guidelines and policies for constitutional specimens.

a) Peripheral Blood

1. Individual with
   - 1.1 primary or secondary amenorrhea, or premature menopause;
   - 1.2 sperm abnormalities – azoospermia or oligospermia;
   - 1.3 clinical features of Turner, Klinefelter, Down, Patau and Edward syndromes;
   - 1.4 ambiguous genitalia;
   - 1.5 a female phenotype and an X-linked recessive condition;
   - 1.6 clinical features of a chromosome instability syndrome, including isolated hematological findings;
   - 1.7 bone marrow transplantation with an opposite sex donor (see also indications for FISH);
   - 1.8 a prenatally diagnosed structural chromosome abnormality, unusual chromosome variant, mosaicism or abnormality with discrepant phenotype/genotype outcome;
   - 1.9 infertility of unknown etiology;
   - 1.10 3 or more pregnancy losses or 2 or more losses where local guidelines permit;.
   - 1.11 clinical features of syndromes with cytogenetic findings such as Roberts, mosaic variegated aneuploidies and immunodeficiency-centromere instability-facial anomaly (ICF) syndromes;
   - 1.12 history of an unexplained stillbirth or neonatal death where it is not possible to study the affected conceptus, dependant on the clinical indications and local policy and resources;
   - 1.13 a suspected chromosome deletion/microdeletion/duplication syndrome (see also section B.2);
   - 1.14 clinically significant abnormal growth: short stature, excessive growth, microcephaly, macrocephaly (see also section B.2);
   - 1.15 dysmorphism (see also section B.2);
   - 1.16 autism spectrum (see also section B.2);
   - 1.17 congenital anomalies (see also section B.2);
   - 1.18 idiopathic mental retardation (see also section B.2);
   - 1.19 developmental delay (see also section B.2).

2. Individual with a significant family history of
   2.1 a chromosome rearrangement;
   2.2 mental retardation of possible chromosomal origin where it is not possible to study the affected individual.

b) Solid Tissues (skin, organs, products of conception, etc.)

1. Individual with:
   1.1 clinically suspected mosaicism in cases known to exhibit tissue specific mosaicism, such as trisomy 8 or i(12p) in Pallister-Killian syndrome;
   1.2 suspected mosaicism based on skin pigmentation variation or a previous ambiguous karyotype.

2. Growth retardation or congenital anomalies in products of conception (POC), abortus tissue, stillbirth/fetal or solid tissue from surgical or post-mortem procedures when a karyotype is unavailable from other sources.

3. It has been suggested that there is an increased risk for subsequent viable trisomy (13, 18, or 21) in women with a previous trisomy (viable or non-viable) detected in a spontaneous abortion (Warburton et al, 2004. Trisomy Recurrence: A Reconsideration Based on North American Data. Am J Hum Genet. 75:376-385). Depending on local policies and financial constraints, cytogenetic analysis of spontaneous abortions may be warranted. However, current prenatal aneuploid screening programs may be a more efficient and economical way to detect the majority of potentially viable trisomic pregnancies.

4. Other exceptional cases may warrant consideration for cytogenetic analysis. Cases should be reviewed on a case by case basis with the laboratory director and a consulting clinical geneticist, if possible.

B.2 Indications for Array Genomic Hybridization technology Testing of Constitutional Specimens

As recommended by the CCMG position statement: Use of array CGH technology in constitutional genetic diagnosis in Canada.

Although it is recognized that microarray technology is an area of transition from classical cytogenetics, the use of array genomic hybridization should become the first line laboratory investigation for the patient who, after a thorough history and physical examination, has unexplained DD/MR, autism, multiple congenital anomalies or dysmorphic features. Routine G-banding and/or FISH analysis may be needed for follow-up confirmation or providing additional information for some cases. Please refer to this document for indications.

B.3 Indications for Fluorescence In Situ Hybridization (FISH) Testing of Constitutional Specimens

1. Individual with:
   - 1.1 a clinical suspicion of a microdeletion syndrome for which established diagnostic testing is available (see also section B.2);
   - 1.2 an increased risk for a microdeletion syndrome because of a positive family history;
   - 1.3 clinical features that suggest mosaicism for a specific chromosomal syndrome;
   - 1.4 a prenatal cytogenetic diagnosis of a standard trisomy in which pathologic examination did not show the characteristic phenotype (not to be used alone for confirmation of prenatally diagnosed mosaicism or chromosome abnormalities other than the most common trisomies);
   - 1.5 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;
   - 1.6 a clinical suspicion of a cryptic subtelomeric rearrangement (see also section B.2.);
   - 1.7 an increased risk for a cryptic subtelomeric rearrangement because of a positive family history;
   - 1.8 rapid aneuploidy detection (RAD) (iFISH or QF-PCR) for newborns suspected with trisomy 13, 18 or 21.
   - 1.9 Follow-up trio analysis of a non-polymorphic copy number variation detected by array genomic hybridization assay.

B.4 Recommendations for the Analysis of Constitutional Studies

Local policies and procedures need to be established for these sample types. Establish at least two cultures when possible.

a) Routine Analysis

Count: minimum of 10 metaphases, routinely.

In some circumstances, as few as 5 metaphases may be examined to confirm the presence or absence of an abnormality.

For clinical conditions where mosaicism is a significant possibility, examination of additional metaphases is required (see also section B.4.b below)

Analyze: minimum of 3 metaphases of the total (if applicable)

Karyotype: minimum of 2 metaphases of the total per cell line.

Use of a formal assessment of band level resolution is recommended. An example reference is as follows: Josifek K *et al*. 1991. Evaluation of chromosome banding resolution: a simple guide for laboratory quality assurance. *Appl Cytogenet* 17:101-105.

Even with use of an identical formal reference for band level resolution there can be large differences between cytogeneticists and between laboratories. Each laboratory therefore needs to establish its own local standards and practices.

Each laboratory should establish its own acceptable level of band resolution for each tissue type and indication. The level of resolution achieved in the analysis should be commensurate with the clinical indication for testing. 550 bands or greater is a reasonable expectation for certain clinical indications, but is recognized to not always be achievable.

b) Mosaicism Analysis

Count: minimum of 25 to 50 metaphases to address the possibility of mosaicism.

For cultured solid tissues, analyze metaphases from two independent primary cultures. An abnormality must be present in at least two primary cultures to diagnose mosaicism.

c) Fluorescence in situ hybridization (FISH) Analysis

i) Microdeletions

Analyze: 10 metaphases in which signals from both diagnostic and control probes are visible. Examine additional metaphases, if any metaphases are discordant, to rule out mosaicism.

A minimum of 50 nuclei, split between two technologists. If there is a discrepancy, an additional 50 nuclei may be examined by a third technologist. If mosaicism is suspect, see below.

ii) Microduplications

Analyze: 10 metaphases in which signals from both diagnostic and control probes are visible. Examine additional metaphases, if any metaphases are discordant, to rule out mosaicism.

A minimum of 50 nuclei, split between two technologists. If there is a discrepancy, an additional 50 nuclei may be examined by a third technologist. If mosaicism is suspect, see below.

iii) Mosaicism

Analyze: 100 to 500 (number based on control values) interphase nuclei from the test specimen.

Analyze interphase nuclei from control specimens (with specific probe and tissue) to establish cut off values for diagnosis of numerical abnormalities.

Interphase FISH in the liveborn for mosaicism may be done as an adjunct to the finding of a few metaphases with the abnormality e.g. a marker from any chromosome identified by SKY or m-FISH.

d) Array-CGH Analysis

Please see CCMG Practice Guidelines for the use of array-CGH technology.

B.5 Recommendations for the Analysis of Individuals with a Suspected Chromosome Instability Syndrome

a) Fanconi Anemia

Analyze: 50 solid-stained metaphases for chromosome breaks in spontaneous and mitomycin C (MMC) or diepoxybutane (DEB) induction lymphocyte cultures established from patient and control specimens.

Analyze 5 G-banded metaphases for a constitutional karyotype.

b) Bloom syndrome

Analyze: 20 metaphases for sister chromatid exchange (SCE) frequency in lymphocyte cultures established from patient and control specimens.

Examine additional metaphases, if analysis suggests the presence of somatic mosaicism e.g. lymphocytes with low and high SCE frequencies in compound heterozygotes.

Analyze: 50 solid-stained metaphases for chromosome breaks and rearrangements if the SCE frequency is negative.

Analyze: 5 G-banded metaphases for a constitutional karyotype.

c) Ataxia telangiectasia and Nijmegen breakage syndrome

Analyze : 50 G-banded metaphases for chromosomes 7 and 14 rearrangements, and chromosome breaks and rearrangements in lymphocyte cultures established from patient and control specimens.

Analyze: 5 G-banded metaphases for a constitutional karyotype.

B.6 Recommendation for Average Turn-Around-Time for Completion of Constitutional Final Reports

Local policies need to be established and suggested guidelines are listed below. At least 90% of all constitutional analyses should have final written reports within the recommended turn-around-time (TAT) listed below.

STAT Lymphocytes	Prelim:	3 days
	Final:	7 days
Lymphocytes (Routine):		4 weeks
Fibroblasts:		6 weeks
Rapid Aneuploidy Detection (iFISH/QF-PCR):		3 days
FISH (Routine, from time of request):		2 weeks
FISH (Custom, from time of request):		4 weeks