CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

CCMG Practice Guidelines for Cytogenetic Analysis

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

Prepared and Submitted by:

CCMG Cytogenetics Committee

June, 2010

Approved by CCMG Board:

July, 2010

Revised by:

CCMG Laboratory Practice Committee

September, 2020

Approved by CCMG Board:

March 4, 2021

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

TABLE OF CONTENTS

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

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 Female phenotype and an X-linked recessive condition;

1.6 Clinical features of a chromosome instability syndrome, including isolated hematological findings;

1.7 A prenatally diagnosed structural chromosome abnormality, rare chromosome variant, mosaicism or abnormality with discrepant phenotype/genotype outcome;

1.8 Infertility of unknown etiology;

1.9 Three or more pregnancy losses or 2 or more losses where local guidelines permit;

1.10 Clinical features of syndromes with cytogenetic findings such as Roberts, mosaic variegated aneuploidies and immunodeficiency centromere instability-facial anomaly (ICF) syndromes;

1.11 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;

1.12 A chromosomal abnormality detected on a cancer specimen suspected to be constitutional rather than somatic in origin;

1.13 An abnormal newborn RAD assay.

2. Individual with a significant family history of:

2.1 A chromosome rearrangement;

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

2.2 Intellectual disability 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 nonviable) detected in a spontaneous pregnancy loss (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 pregnancy loss 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 Genomic Microarray Analysis Testing of Constitutional Specimens

The use of genomic microarray analysis should be the first line laboratory investigation for a patient who, after a thorough history and physical examination, has unexplained developmental delay/intellectual disability, autism, multiple congenital anomalies and/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 see CCMG Practice Guidelines for Genomic Microarray Testing.

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

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

1. Individual with:

1.1 A clinical suspicion of a microdeletion syndrome for which established diagnostic testing is available may be warranted, however it would be advantageous to perform genomic microarray analysis as a first line investigation rather than FISH or MLPA analysis;

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 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;

1.5. An increased risk for a cryptic subtelomeric rearrangement because of a positive family history.

2. Rapid aneuploidy detection (RAD) (iFISH or QF-PCR) for newborns suspected with trisomy 13, 18 or 21 or ambiguous genitalia

3. Familial follow-up of a copy number variation detected by genomic microarray analysis which was interpreted as clinically significant or of uncertain clinical significance.

4. Bone marrow transplantation (BMT) with an opposite sex donor.

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

Karyotype: minimum of 2 metaphases per cell line.

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

Use of a formal assessment of band level resolution is recommended (Josifek K et al. 1991. Evaluation of chromosome banding resolution: a simple guide for laboratory quality assurance. Appl Cytogenet 17:101-105; Stallard R, Johnson W 1983. Nonsubjective method for estimating the resolution of banded chromosomes. Am J Hum Genet 35:155A.; Zabawski J et al. 2005 Use reference bands to accurately estimate ISCN band levels 400, 500, and 850. J Assoc Genet Technol 31:9–13).

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

1. 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 suspected, see below.

2. Microduplications

Analyze: 10 metaphases to determine if a duplication identified by a molecular method such as genomic microarray, quantitative PCR or multiplex ligation-dependent probe amplification is located in tandem or inserted elsewhere in the genome when clinically indicated. Analysis of at least 50 nuclei is recommended if the metaphases are normal or ambiguous to further attempt to confirm a tandem duplication. A positive control for the probe must be used for familial studies (i.e. the patient with the duplication detected by genomic microarray analysis or other molecular technique).

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

3. RAD iFISH

Analyze: 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 suspected, see below.

4. Mosaicism

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

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

5. Bone Marrow Transplant with Opposite Sex Donor

Analyze: A minimum of 200 nuclei, split between two technologists. If there is a discrepancy, an additional 100 nuclei may be examined by a third technologist.

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.

CCMG-CCGM National Office
4 Cataraqui Street, Suite 310
Kingston, Ontario K7K 1Z7
(T) 613-507-8345 (F) 1-866-303-0626
(E) info@ccmg-ccmg.org (W) www.ccmg-ccgm.org

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