Canadian College of Medical Geneticists guidelines for the indications, analysis, and reporting of cancer specimens

A.J. Dawson PhD, J. McGowan–Jordan PhD,† J. Chernos PhD,‡ J. Xu PhD,§ J. Lavoie PhD,|| J.C. Wang PhD,# M. Steinraths MD,** and S. Shetty PhD††*

ABSTRACT

The Canadian College of Medical Geneticists (CCMG) is a national organization of medical and laboratory geneticists. The mission of the CCMG is to establish and maintain high-quality professional and ethical standards for medical genetics services in Canada and to help to ensure that service of the highest quality is delivered to the Canadian public.

Cancer cytogenetics is one of the sections of practice of the CCMG. The CCMG Cytogenetic Committee has, therefore, put forward guidelines to provide oncologists and CCMG cytogeneticists with a comprehensive review of the cytogenetic diagnostic tests that are recommended as a minimum standard of care for tumours of hematopoietic and lymphoid tissues and for tumours of soft tissue and bone. The guidelines were approved by the CCMG board of directors in June 2010.

KEY WORDS

Cytogenetics, cancer, hematopoietic, lymphoid, tumours

1. INTRODUCTION

The Canadian College of Medical Geneticists (CCMG) guidelines are compiled from various sources and are based primarily on expert opinion and descriptive papers that demonstrate the value of cytogenetics in the diagnosis, prognosis, and management of neoplasia. The indications for cancer cytogenetics are not all-inclusive and may be extended where there is local interest. The guidelines that follow are minimum recommendations only. They are subject to the discretion of the laboratory director and to the requirements, capabilities, and funding of the local cytogenetic laboratory. The guidelines are based on the information that was available at the time of writing, and they will change with advances in cytogenetic knowledge. Standard of practice—that is, in-house protocols—should be established for the processing of tissue and cell types, culture setup, analysis, quality control, and turnaround times based on the reason for the cytogenetic testing (for example, initial diagnosis or follow-up) and the clinical utility of the cytogenetic information (for example, diagnosis, prognostic value, or selection of therapy). All cytogenetic findings should be interpreted in the context of clinical, morphologic, and other laboratory findings whenever possible. Molecular assays are also available for many of the neoplastic disorders described in the guidelines, and the implementation and use of specific molecular tests is based on the funding and capability of the local genetic centre. In addition, clinical microarray platforms for oncology are currently being developed and evaluated by the Cancer Cytogenomics Microarray Consortium (http://www.urmc.rochester.edu/ccmc/) clinical trial group.

2. GUIDELINES

2.1 Indications for Cancer Cytogenetic Investigations

A complete cytogenetic analysis of bone marrow should be performed

- at the time of initial evaluation to establish the cytogenetic profile, and
- at intervals thereafter as clinically indicated to detect persistence of an abnormal clone or evidence of genetic or clonal evolution.

A standard of practice for the cancer cytogenetics of specific neoplastic disorders, including fluorescence *in situ* hybridization (FISH), should be established at each genetic centre. The neoplastic disorders listed in the rest of this subsection accord with the 2008 World Health Organization classification of tumours of hematopoietic and lymphoid tissues and of tumours of soft tissue and bone [1]. The

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cytogenetic and FISH requirements are listed for each neoplastic disorder.

2.1.1 Chronic Myeloproliferative Neoplasms

Chronic Myelogenous Leukemia: Karyotype for t(9;22)(q34;q11.2) at diagnosis and FISH with probes corresponding to BCR/ABL1 as required to confirm diagnosis in unusual cases or cases with no or few metaphases (or to establish the signal patterns, detect cryptic rearrangements, and so on). Follow-up investigation may be indicated for staging purposes or to monitor the effect of treatment.

Karyotype or FISH may be indicated at diagnosis for these disorders:

- Polycythemia vera
- Chronic idiopathic myelofibrosis
- Essential thrombocythemia
- Chronic eosinophilic leukemia
- Chronic neutrophilic leukemia

2.1.2 Myeloid and Lymphoid Neoplasms Associated with Eosinophilia

At diagnosis, karyotype or FISH, or both, is required to detect abnormalities of *PDGFRB* (5q31~q33) or *FGFR1* (8p11), and FISH is required to detect cryptic abnormalities of *PDGFRA* (4q12).

2.1.3 Myelodysplastic or Myeloproliferative Neoplasms

Chronic Myelomonocytic Leukemia: Karyotype or FISH, or both, for BCR/ABL1 to rule out t(9;22) and *PDGFRB* (5q31~35), and FISH for *PDGFRA* (4q12) if eosinophilia is present.

Atypical Chronic Myeloid Leukemia: Karyotype or FISH, or both, for *BCR/ABL1* to rule out t(9;22).

Juvenile Myelomonocytic Leukemia: Karyotype at diagnosis.

2.1.4 Myelodysplastic Syndromes

Karyotype at diagnosis, especially in the patient eligible for a bone marrow transplant. Follow-up investigation may be indicated at disease progression and after treatment.

2.1.5 Acute Leukemia, Myeloid and Lymphoid

Karyotype all cases, with FISH as indicated based on chromosome morphology and clinical and pathologic features. If an abnormality is present, a follow-up after treatment or at relapse may be indicated. If an abnormal clone is not detected, re-investigation at relapse may be indicated in a second attempt to detect a disease-related clone.

2.1.6 Mature B-Cell Neoplasms

Chronic Lymphocytic Leukemia: Fluorescence *in situ* hybridization to detect abnormalities such as +12, del(13)(q14), or deletion of *ATM* and *TP53* can be performed. The specific FISH loci to be tested and the need for conventional karyotyping would depend on the policy of the local centre.

Plasma Cell Myeloma: At a minimum, FISH to detect abnormalities such as t(4:14)(p16;q32) and deletion of *TP53* should be performed. The specific FISH loci to be tested and the need for conventional karyotyping will depend on the policy of the local centre. Investigations should preferentially target the analysis of plasma cells.

Karyotype or FISH, or both, may be appropriate at diagnosis in selected cases, in consultation with the pathologist or clinician, for these disorders:

- Marginal zone lymphoma or mucosa-associated lymphoid tissue lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
- Burkitt lymphoma

2.1.7 T-Cell Neoplasms

Karyotype or FISH, or both, may be appropriate at diagnosis in selected cases, in consultation with the pathologist or clinician, for these disorders:

- T-Cell prolymphocytic leukemia
- Hepatosplenic T-cell lymphoma
- Anaplastic large cell lymphoma

2.1.8 Solid Tumours

Karyotype or FISH, or both, may be appropriate at diagnosis for small-round-cell tumours of childhood, selected sarcomas, lipomatous tumours, and other tumours, in consultation with the pathologist or clinician. In consultation with the pathologist or clinician, FISH may also be used for the diagnosis and prognosis of some carcinomas—for example, bladder, prostate, breast.

2.2 Recommendations for the Processing of Cancer Cytogenetic Specimens

Specimens include bone marrow, blood, lymph node, solid tumour, needle aspirates, fluids, and effusions. Many methods are used for culturing and harvesting cancer specimens. Here are some recommendations, based in part on the American College of Medical Geneticists (ACMG) standards and guidelines [2]:

- Bone marrow is the tissue specimen of choice for the analysis of suspected hematologic disorders, including aplastic anemia and chronic myeloproliferative disorders. When bone marrow is not available, unstimulated peripheral blood may provide useful information if sufficient immature cells are present. Culture conditions should be

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optimized for the specific hematologic disorder suspected. A bone core biopsy may sometimes be the only option. A bone core biopsy should be mechanically or enzymatically minced to yield cell suspensions, which are then cultured as for bone marrow aspirates.

- Cultured (overnight or short-term) bone marrow harvests are preferred for analysis. A direct harvest may also provide metaphases for analysis. For acute leukemias, unstimulated short-term cultures are recommended. If sufficient specimen is received, at least 2 cultures should be initiated, 1 of which should be a 24-hour or overnight culture.
- Blood specimens produce better results when they are harvested after short-term to 48-hour culture, rather than directly.
- In most cases, it is preferable to analyze unstimulated bone marrow or blood specimens, but to encourage clonal divisions in specific disorders, the culture medium may be supplemented with B-cell or T-cell mitogens (for mature B- and T-cell disorders) and other culture additives such as hormones and growth factors. However, unstimulated cultures should always be examined in these cases, because additives may mask an abnormal clone.

Mature B-Cell Neoplasms (for example, chronic lymphocytic leukemia, plasma cell myeloma): Although there is no consensus on this point, some laboratories have had success in identifying abnormal clones with the addition of B-cell mitogens.

Mature T-Cell Neoplasms (for example, T-cell leukemia or lymphoma): Addition of T-cell mitogens may be helpful.

- Lymph node is the tissue of choice for the analysis of suspected lymphoma. Lymph nodes should be disaggregated mechanically or enzymatically, or both, to create a suspension of single cells, which is then cultured and harvested as for bone marrow specimens.
- Effusions and fluids are harvested directly or after 24-hour culture.
- Solid tumour tissue should be disaggregated mechanically or enzymatically, or both, to create a suspension of single cells and small cell clusters. Whenever possible, cells should be harvested within 1 week of establishing the culture of a solid tumour. Tumours that have been cultured for longer periods may be overgrown by normal fibroblasts. Normal results should be interpreted with caution and should include a statement that mentions the length of time in tissue culture.
- The failure rate for bone marrow and neoplastic blood specimens should not exceed 10%. Failures include both culture failures and inadequate specimens.

2.3 Recommendation for Chromosome Analysis of Cancer Specimens

2.3.1 General

- Consultation with the clinician or pathologist, or both, is recommended to assist in the analysis and interpretation of cancer specimens whenever possible. The results should be correlated with other laboratory and clinical findings.
- Cytogenetic follow-up may be indicated in specific circumstances and at appropriate intervals to evaluate disease progression or the effect of treatment.
- The recommendations that follow are minimum chromosome analysis guidelines. The extent and focus of the analysis will vary with the clinical situation. Fluorescence *in situ* hybridization or molecular methods may replace or supplement chromosome analysis in some situations. It is recommended that laboratories develop local or regional testing algorithms, based on the availability of FISH, molecular diagnostics, and other laboratory tests.
- The numbers of cells to be analyzed at diagnosis are minimum numbers, which are supported by descriptive studies [3,4]. Some disorders more frequently have a low percentage of abnormal cells [3]. For diagnoses of those disorders, analysis of more cells may be warranted. In addition, if the presence of normal cells or clonal evolution is significant, more cells may have to be examined or analyzed.
- For chromosome analysis, the metaphases that are selected should represent the range of chromosome morphology on the slides—that is, select metaphases with poor chromosome morphology as well as those with good morphology.
- A normal cytogenetic result at diagnosis generally does not warrant further cytogenetic analysis after treatment and remission. It should be noted, however, that cytogenetic analysis may sometimes be requested to rule out a therapy-related abnormality (for example, myelodysplastic syndrome), rather than disease recurrence.
- For determination of engraftment status, molecular methods are preferred for the determination of recipient versus donor cells. For determination of relapse, cytogenetic G-band analysis or interphase FISH analysis, or both, may be warranted, depending on the proportion of donor to recipient cells and in consultation with the pathologist or clinician.

2.3.2 Chronic Myelogenous Leukemia and Other Chronic Myeloproliferative Diseases

At Diagnosis: Examine sufficient metaphases (minimum of 10) to confirm the presence of an

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abnormal clone at diagnosis and for pre-transplantation assessment. When disease transformation is suspected, examine additional metaphases to rule out secondary abnormalities. Analyze at least 20 metaphases if normal.

Post-Treatment Monitoring: Examine sufficient metaphases (minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least 1 metaphase per stem line and significant side line. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

2.3.3 Acute Leukemia and Myelodysplasia

At Diagnosis: Analyze enough metaphases (minimum of 10) to confirm the presence of an abnormal clone (per the International System for Chromosome Nomenclature, 2009) [5]. If only normal metaphases are found, or if the presence of clonal evolution is significant, analyze or examine at least 20 metaphases.

Exception: In cases of confirmed pediatric pre–B-cell acute lymphoblastic leukemia (ALL), identification of the abnormal clone by more extensive analysis is warranted.

Post-Treatment Monitoring: Examine sufficient metaphases (minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least 1 metaphase per stem line and significant side line. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

2.3.4 Mature B-Cell Neoplasms, Malignant Lymphoma, and Solid Tumours

At Diagnosis: Analyze sufficient metaphases (minimum of 10) to identify an abnormal clone, or 20 metaphases if normal.

Post-Treatment Monitoring: Examine sufficient metaphases (minimum of 10) to confirm the presence of the original abnormal clone. If normal, examine at least 25 metaphases.

Karyotype at least 1 metaphase per stem line and significant side line. A normal metaphase (when present) should be printed; a normal karyotype is recommended.

2.4 Recommendations for FISH Analysis of Cancer Specimens

2.4.1 General

- In some situations, FISH is helpful in patient management because, compared with chromosome analysis, it provides greater sensitivity or a more rapid result, or both. The extent of analysis will depend on local resources and the availability of molecular diagnostics. The guidelines that follow are for FISH applications that have been demonstrated to be of clinical value. (For details, consult the ACMG standards and guidelines [2].)
- On interphase nuclei, FISH can be used for chromosome enumeration and for some types of rearrangement detection. Metaphase FISH can assist in the identification of markers and unusual or variant chromosome rearrangements.
- Fluorescence *in situ* hybridization can be performed on any source of fresh, frozen, fixed, and paraffin embedded tissue or cells, including touch preparations and cytology slides.
- All probes should be validated and cut-off values determined in-house before clinical use. (Consult the ACMG standards and guidelines [2].)
- The limitations of FISH analysis must be stated in the report when appropriate.
- For the detection of translocations in interphase nuclei, probe sets that result in an extra signal with a single fusion or with double fusions should be used whenever possible.
- In general, FISH signals should be scored as instructed by the manufacturer of the probe or analysis kit.
- All interphase FISH analyses should be performed by at least 2 qualified individuals. If results are discrepant, additional nuclei may be examined by a third technologist.

2.4.2 Sex Chromosomes

After bone marrow transplantation involving an opposite-sex donor, use dual-color probes to examine a minimum of 200 interphase nuclei or 50 metaphase cells for the X and Y chromosome [6,7].

2.4.3 Numerical Abnormalities

- At diagnosis, FISH with centromeric probes can be used to examine poor-quality metaphases or interphase nuclei for numerical chromosome abnormalities. This approach is particularly useful when the abnormality being sought has prognostic value—for example, –7 in myelodysplastic syndrome or acute myelogenous leukemia, and +4/+10/+17 in ALL.
- At follow-up of a patient with a known numerical abnormality, interphase FISH can be used to detect residual disease.
- Gene amplification can be detected by FISH analysis as multiple fluorescence signals (more than the normal diploid copy number)—for example, HER2/*neu* (*ERBB2*) in breast carcinoma or *NMYC* in neuroblastoma.

2.4.4 Translocation and Deletion Detection

- Fluorescence *in situ* hybridization can be used to detect cryptic rearrangements such as *ETV6/RUNX1* fusion in ALL, *FIP1L1–PDGFRA* fusion,

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or *BCR/ABL1* rearrangements in chronic myeloproliferative neoplasms.
- Fluorescence *in situ* hybridization can be used to clarify ambiguous or complex rearrangements, or to assist in clarifying rearrangements when morphology is poor (for example, to confirm the involvement of mixed lineage leukemia in an 11q23 rearrangement)
- Interphase FISH can be useful at follow-up to detect specific structural abnormalities such as t(9;22) [8]. Analyze a minimum of 200 nuclei.

2.4.5 Marker Identification

In individual cases, identification of a marker chromosome may be clinically significant. Paint probes or multicolor FISH techniques can be used in an attempt to identify markers.

2.5 Recommendation for Turnaround Time to Completion of Reports

Local policies have to be established, and guidelines for those policies are suggested below. Final written reports for at least 90% of all neoplastic analyses should be completed within the recommended turnaround times listed below.

- For cytogenetics and FISH, and depending on the clinical indication (for example, acute promyelocytic leukemia for treatment purposes), a preliminary result should be reported within 3–5 days, with the final written report being completed within 2 weeks.
- For routine cytogenetics or FISH, or both, at diagnosis for ALL, acute myelogenous leukemia, and chronic myelogenous leukemia, final results should be reported within 2 weeks. Results for other neoplastic disorders may be reported within 3 weeks.
- For routine cytogenetics or FISH, or both, at follow-up, final results should be reported within 3 weeks.

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4. CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to declare.

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Correspondence to: Angelika J. Dawson, Cytogenetics Laboratory, 685 William Avenue, Winnipeg, Manitoba R3E 0Z2.
E-mail: adawson@hsc.mb.ca

* Cytogenetics Laboratory, Diagnostic Services Manitoba, Winnipeg, MB. (Chair, CCMG Cytogenetic Committee 2009–2010)

† Cytogenetics Laboratory, Children's Hospital of Eastern Ontario, Ottawa, ON. (Past Chair, CCMG Cytogenetic Committee 2009–2010)

‡ Cytogenetics Laboratory, Alberta Children's Hospital, Calgary, AB. (Board Representative, CCMG Cytogenetic Committee 2009–2010)

§ Cytogenetics Laboratory, McMaster University, Hamilton, ON.

|| McGill University Cytogenetics, Montreal Children's Hospital, Montreal, QC.

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[#] Cytogenetics Laboratory, Health Sciences Centre, London, ON.
** Division of Medical Genetics, Victoria General Hospital, Victoria, BC.
†† ARUP Laboratory, Salt Lake City, UT, U.S.A. (Project Leader, CCMG Cytogenetic Committee 2009–2010)

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