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ACMG TECHNICAL STANDARD

Section E6.1–6.6 of the American College of Medical Genetics and Genomics (ACMG) Technical Laboratory Standards: Cytogenomic studies of acquired chromosomal abnormalities in neoplastic blood, bone marrow, and lymph nodes

Yassmine Akkari[1], Linda B. Baughn[2], Annette Kim[3], Ender Karaca[4,5], Gordana Raca[6,7], Lina Shao[3], Fady M. Mikhail[8]; on behalf of the ACMG Laboratory Quality Assurance Committee[9,*]

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ABSTRACT

Cytogenomic analyses of acquired clonal chromosomal abnormalities in neoplastic blood, bone marrow, and/or lymph nodes are instrumental in the clinical management of patients with hematologic neoplasms. Cytogenetic analyses assist in the diagnosis of such disorders and can provide important prognostic information. Furthermore, cytogenetic studies can provide crucial information regarding specific genetically defined subtypes of these neoplasms that may have targeted therapies. At time of relapse, cytogenetic analysis can confirm recurrence of the original neoplasm, detect clonal disease evolution, or uncover a new unrelated neoplastic process. This section deals specifically with the technical standards applicable to cytogenomic studies of acquired clonal chromosomal abnormalities in neoplastic blood, bone marrow, and/or lymph nodes. This updated Section E6.1-6.6 supersedes the previous Section E6 in Section E: Clinical Cytogenetics of the American College of Medical Genetics and Genomics Technical Standards for Clinical Genetics Laboratories.

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*Correspondence: ACMG. *Email address:* documents@acmg.net
Affiliations are at the end of the document.

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6.1. Introduction

The cytogenomic assessment of bone marrow and lymph node specimens is essential in the evaluation of hematologic malignancies and has long been implemented in the clinical setting. In Sections E6.1-E6.6 herein, we update the 2016 document[1] and discuss the general considerations, pre-analytical, analytical, and post-analytical components, as well as provide guidance for the G-banded chromosome studies, fluorescence in situ hybridization (FISH), and chromosomal microarray analysis (CMA) of hematologic disorders. The goal is to provide practice consistency across laboratories using expert opinions and published data.

6.2. General considerations

6.2.1 Cytogenomic analyses of acquired clonal chromosomal abnormalities in neoplastic blood, bone marrow, and/or lymph nodes are instrumental in the clinical management of patients with hematologic neoplasms. Cytogenetic analyses assist in the diagnosis of such disorders and can provide important prognostic information.[2-6] Furthermore, cytogenetic studies can provide crucial information regarding specific genetically defined subtypes of these neoplasms that may have targeted therapies. At time of relapse, cytogenetic analysis can confirm recurrence of the original neoplasm, detect clonal disease evolution, or uncover a new unrelated neoplastic process. In some circumstances, cytogenetic studies may be useful in post-treatment evaluation to identify persistent disease (with or without clonal evolution) or to document cytogenetic remission.

6.2.2 These cytogenetic analyses include conventional G-banded chromosome studies, FISH, and/or CMA. In addition, there is a growing overlap between cytogenetic and molecular genetic studies and several novel methodologies fall between these traditional boundaries and complement traditional cytogenetic methods. Laboratories should work closely with oncologists and pathologists to determine the order of testing required to obtain relevant cytogenetic information in a cost- and time-efficient manner.

6.2.3 Laboratories offering cytogenetic analyses for hematologic neoplasms should be familiar with the various chromosomal abnormalities associated with these different malignancies and their clinical significance. The laboratory should provide a robust analytical and interpretative service for the various hematologic neoplasms. All results should be, to the extent possible, interpreted in the context of the clinical, pathologic, and molecular findings.[2-7]

6.2.4 Sample processing, analytical variables, and turnaround time (TAT) should be determined by the laboratory based on the indication for cytogenetic referral (eg, initial diagnosis vs follow-up studies, pre- vs post-transplant studies, and lymphoid vs myeloid malignancies) and the clinical application of the cytogenetic results (eg, selection of therapy).

6.2.5 Molecular genetics analyses are essential for the diagnosis of some hematologic neoplasms, and several clinically significant molecular variants, not detectable by cytogenetic analyses, provide important diagnostic and prognostic information. However, molecular methodologies used to detect genomic aberrations such as single-nucleotide variants (SNVs), indels, RNA fusions, or internal tandem duplications are outside the scope of this document.

6.2.6 For quality assurance, the laboratory should monitor the numbers and types of hematologic neoplasms received, percentage of cases with abnormal results, cell culture success rate, success rate of FISH and CMA studies, TAT, and correlation of FISH and CMA data with G-banded chromosome analysis results. In addition, correlation with clinical and pathologic findings, as well as additional structural chromosomal abnormalities detected by other molecular methods should be documented whenever applicable.

6.3. Methods

6.3.1 These technical laboratory standards were informed by a review of the literature and current guidelines. Resources consulted included PubMed, American College of Medical Genetics and Genomics (ACMG) technical standards, National Comprehensive Cancer Network (NCCN), and Children's Oncology Group, as well as the current World Health Organization (WHO) classification and International Consensus Classification (ICC). The workgroup members also used their expert opinion and empirical data to inform their recommendations. Any conflicts of interests for workgroup members are listed at the end of the paper. The ACMG Laboratory Quality Assurance Committee reviewed the document providing further input on the content, and a final draft was presented to the ACMG Board of Directors for review and approval to post on the ACMG website for member comment. Upon posting to the ACMG website, an email and link were sent to all ACMG members inviting participation in the 30-day open comment process. All members' comments and additional evidence received were assessed by the authors, and these recommendations were incorporated into the document as deemed appropriate. Member comments and author responses were reviewed by representatives of the ACMG Laboratory Quality Assurance Committee and the ACMG Board of Directors. The final document was approved for publication by the ACMG Board of Directors.

6.3.2 The 2022 WHO and ICC classifications of hematologic malignancies were published during the final stages of development of these technical standards.[2-6] The workgroup made recommendations that are informed by both classifications as they pertain to the cytogenetic analyses of hematologic malignancies. Significant differences between the 2 classifications regarding the inclusion of cytogenetic data were noted and incorporated.

6.4. Pre-analytical considerations

6.4.1. Specimen type and collection

6.4.1.1 The specimen type and culture techniques utilized should optimize the probability of detecting an abnormal clone.

6.4.1.2 The following list includes fresh specimen types that are appropriate for cytogenetic analysis of hematologic neoplasms:

a. Bone marrow aspirate is the preferred specimen for most hematologic neoplasms.
b. Bone marrow core biopsy is an option in cases with a dry tap and will require special processing (see Section 6.4.2.2).
c. Bone marrow smear, core biopsy touch imprint preparations can be used for interphase FISH analysis, if an inadequate bone marrow aspirate is obtained.
d. Peripheral blood may yield informative results when it contains sufficient neoplastic cells that exceed the analytical sensitivity of the assay. In general, peripheral blood G-banded chromosome analysis is appropriate when sufficient clonal abnormal metaphase cells are obtained.
e. Lymph node biopsy or biopsy from a suspected lymphoid mass are also appropriate specimen types.
f. Body fluids (eg, cerebrospinal fluid, pleural fluid, and peritoneal fluid), if involved in the neoplastic process and sufficiently cellular, may be used.
g. Extramedullary leukemia (myeloid sarcoma, chloroma) tissue biopsy is appropriate in patients with extramedullary disease.

6.4.1.3 Specimens should be collected under sterile conditions in sodium heparin tubes or transport media with sodium heparin for chromosome and/or FISH analyses (lithium heparin tubes are not recommended). Sodium heparin prevents coagulation without interfering with cell culture and without diminishing the quality of the preparation. The optimal concentration of sodium heparin should be 20 IU/mL of specimen (per either bone marrow volume alone or per total volume of bone marrow and transport medium combined).[8] EDTA tubes may be used for assays that require genomic DNA (gDNA) or RNA extraction; however, EDTA has been shown to impair cell growth in culture. It should be noted that heparin is a polymerase chain reaction (PCR) inhibitor and should be avoided if amplification of the DNA is anticipated. Of note, any downstream RNA-based studies will require special specimen handling.[9]

6.4.1.4 The volume of bone marrow available will differ for adults and children. An approximate specimen volume of 1 to 3 mL should be requested. During specimen procurement, several draws are likely to be collected, typically becoming progressively more hemodilute. Because the first draw typically contains more neoplastic bone marrow cells, it is recommended that cytogenetics receive the first or second draw whenever possible.

6.4.1.5 Specimens should be received by the laboratory as soon as possible, ideally within 24 hours (for optimal plasma cell neoplasms (PCNs) specimen processing, see Section 6.4.2.3c).[8] Also, it is recommended that specimens be maintained at ambient temperature during transit. Extreme temperatures should be avoided.

6.4.2. Specimen processing and quality

6.4.2.1 The laboratory should process the specimen as soon as possible after it is received. The methods that will be used to analyze the specimen should be determined before processing whenever possible. If chromosome analysis is requested, cell culture will be required. If FISH and/or CMA analyses are requested, a portion of the specimen can be used for direct harvest of interphase cells and/or gDNA extraction. If the diagnosis is unclear at the time of specimen processing, the laboratory (in consultation with the treating physician) may still want to perform direct harvest of interphase cells and/or gDNA extraction but put the FISH and/or CMA analyses on-hold until a more definitive diagnosis is available. This is important to preserve the integrity of the specimen used for FISH and/or CMA. Interphase FISH analysis can be performed on the cultured fixed cell pellet; however, this has the potential of introducing culture bias.

6.4.2.2 If a bone marrow core biopsy, or other solid tissue, is obtained, it should be disaggregated to generate a cell suspension. This can be achieved by mechanical mincing and/or enzymatic digestion using collagenase. Culture conditions are the same as those for a bone marrow aspirate.

6.4.2.3 Cell culture conditions should be optimized for the specific hematologic neoplasm suspected:

a. Acute leukemias, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and acute leukemias of ambiguous lineage: unstimulated short-term cultures are recommended. If sufficient specimen is received, at least 2 cultures should be initiated, including direct, overnight, and/or 24-hour cultures. In pediatric ALL, an additional unstimulated 48-hour culture can be useful in the identification of an abnormal clone. The seeding density is usually 1 to 2 million cells per mL of medium.[8]
b. Myelodysplastic neoplasms/syndromes (MDS) and myeloproliferative neoplasms (MPN): Same as acute leukemias.

c. Plasma cell myeloma (also referred to as multiple myeloma [MM]) and other PCNs: Unstimulated 24-hour and B cell mitogens (eg, IL-4) stimulated cultures may be performed.[10] The clinical utility of G-banded chromosomal analysis in PCNs will be discussed in a subsequent section (see Section 6.5.4.5). Of note, establishing bone marrow cultures from patients with previously diagnosed and treated PCN may also allow the detection of concurrent de novo or therapy-related myeloid neoplasms. For FISH and/or CMA analyses, if the bone marrow plasma cell percentage (as determined by aspirate count or flow cytometry) is below a laboratory-validated cutoff value, CD138+ (syndecan-1) plasma cell enrichment is recommended.[11,12] Each laboratory needs to establish its own cutoff value for plasma cell enrichment.[13] Specimens of PCNs received for FISH analysis should be processed as soon as possible, preferably within 24-hours, to ensure optimal plasma cell recovery.[13] Surface expression of CD138 has been shown to be reduced when sample processing is delayed.[14,15] Alternative approaches such as flow sorting using additional surface antibodies such as CD319 or CD229 can be considered for improved plasma cell yield.[16,17]

d. Mature lymphoid neoplasms:
   1) Peripheral blood and bone marrow: depending on the immunophenotype, additional cultures with B or T cell mitogens may be helpful.
      - Chronic lymphocytic leukemia (CLL) and other mature B cell neoplasms: CpG-oligonucleotide cell stimulation is recommended and has been shown to enhance the detection of clonal chromosomal abnormalities.[18,19]
      - Well differentiated T cell disorders (eg, T cell leukemias, T cell lymphoma, Sézary syndrome, and mycosis fungoides): T cell mitogens, such as phytohemagglutinin, may be helpful.
   2) Fresh lymphoid tissue:
      - Disaggregation of lymphoid tissues into single-cell suspension is necessary before culture initiation. The lymphoid cells in most tissues are readily disaggregated by mechanical means, such as mincing with scalpels or curved scissors. The use of these methods is often advantageous if the tissue is easily dissociated because it will keep the loss of cells to a minimum and may help minimize stromal contamination as stromal cells are often locked in fibrous connective tissues. If cells are not readily released by mechanical means, enzymatic digestion may be necessary. When using enzymatic digestion, the tissue must first be minced and then incubated with the enzyme solution (eg, collagenase) for 20 minutes to 16 hours, depending on how quickly cell release occurs.
      - Disaggregated cells are cultured in suspension using appropriate supportive growth medium. Tumor cells may spontaneously divide; however, mitogens may be used for lymphoid disorders to encourage proliferation of the desired cell type.
      - Depending on the amount of available tissue, a combination of direct, 24-hour, and/or 48-hour cultures are most often utilized for lymphoid disorders.
   3) Formalin-fixed paraffin embedded (FFPE) tissue: FFPE tissue is acceptable for FISH and CMA analysis (see Sections 6.5.2 and 6.5.3).

6.5. Analysis

6.5.1. Conventional G-banded chromosome analysis

6.5.1.1 Cell selection: Metaphase cells should not be selected for analysis solely on the basis of good chromosome morphology. In general, the technologist should select an area of the slide to begin the analysis and then examine metaphase cells as they appear consecutively in the microscope field, only skipping cells for which extremely poor morphology precludes chromosome identification. This technique can also be performed using automated metaphase finders by examining metaphase cells consecutively captured by the system. Sufficient cells should be analyzed or examined to maximize the detection of an abnormal clone and establish the clonality of the abnormality found (please see Section 6.5.1.3 below). For each abnormal clone identified, clonal cells with the best chromosome morphology should be analyzed, captured, and karyotyped to provide the most accurate breakpoint assignment. When cells are skipped because of poor morphology, it is important to attempt to count the number of chromosomes. This is particularly true for hyperdiploid and hypodiploid B-lineage ALLs (B-ALLs) or PCNs. In addition, attempts should be made to identify possible structural chromosomal abnormalities, particularly if the disease under consideration is associated with a specific recurring abnormality (eg, the t(9;22) in chronic myeloid leukemia [CML]).

6.5.1.2 Number of cells evaluated: The number of metaphase cells analyzed vs the number of cells counted or scored for a particular chromosome/abnormality should be appropriate for the type of the study (eg, initial diagnosis or follow-up studies) and the purpose of the study (eg, detection of residual disease or response to therapy, monitoring for clonal evolution, or monitoring of allogeneic transplant engraftment) as discussed in Section 6.5.1.3 below.

6.5.1.3 Initial diagnostic studies:

a. Analysis: Analyze a minimum of 20 cells from unstimulated cultures. For the mature B and T cell disorders, adequate representation of cells analyzed from a combination of both unstimulated and mitogen-stimulated cultures may be appropriate as described. Unstimulated CLL cultures infrequently yield CLL-related clonal chromosomal abnormalities; however, they can also reveal MDS/AML-related clonal abnormalities because some of these patients may have

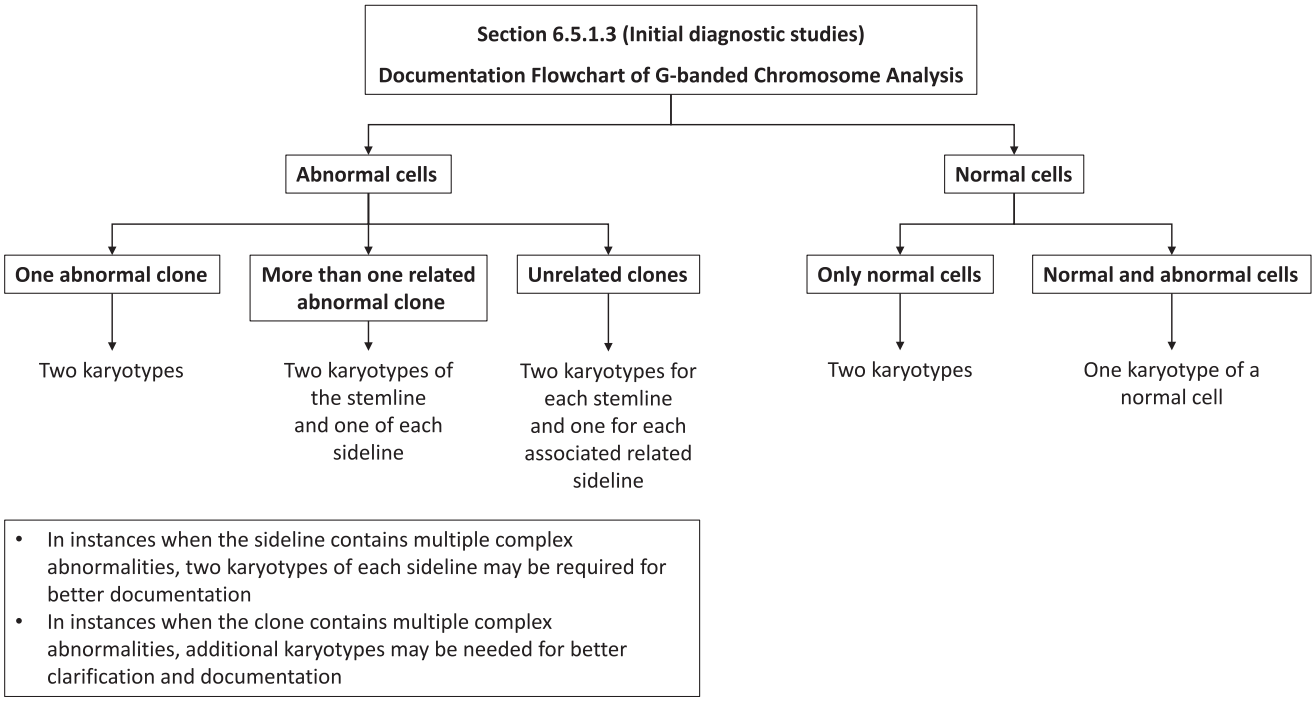

Figure 1 Documentation flowchart of G-banded chromosome analysis in initial diagnostic studies (Section 6.5.1.3).

concurrent de novo or therapy-related myeloid neoplasms. Similarly, unstimulated 24-hour PCN cultures can reveal clonal abnormalities from concurrent myeloid neoplasms.

b. Documentation (Figure 1):
   - For the abnormal cells:
     - If only 1 abnormal clone is present: 2 karyotypes.
     - If more than 1 related abnormal clone is present: 2 karyotypes of the stemline and one of each sideline.
     - If unrelated clones are present: 2 karyotypes for each stemline and 1 for each associated related sideline.
     - In instances when the sideline contains multiple complex abnormalities, 2 karyotypes of each sideline may be required for better documentation.
     - In instances when the clone contains multiple complex abnormalities, additional karyotypes may be needed for better clarification and documentation.
   - For the normal cells:
     - If only normal cells are present: 2 karyotypes.
     - If normal and abnormal cells are present: 1 karyotype of a normal cell.

6.5.1.4 Follow-up studies of patients who have had a previous cytogenetic study: for the following analytic guidelines, it is assumed that the laboratory has documentation of the patient's previous cytogenetic results. If the study has been performed elsewhere and there is minimal information available, it is recommended that, except for patients seen for the first-time post-transplant, the analysis must be considered the same as an initial diagnostic workup (see Section 6.5.1.3).

I. Patients who have not undergone allogeneic hematopoietic cell transplantation:

a. Analysis: Analyze 20 metaphase cells. If all cells are normal, additional cells may be scored for a specific abnormality by G-banded chromosome or FISH analysis if pathology suggests a specific karyotypic abnormality. For some patients, follow-up cytogenetic study is ordered to rule out a therapy-related malignancy (eg, MDS) rather than disease recurrence.

b. Documentation:
   - For cases with both normal and abnormal cells or only abnormal cells:
     - One karyotype of a normal cell, if such a karyotype was not documented in a previous study by the laboratory; otherwise, 1 normal metaphase spread.
     - One or 2 karyotypes from each abnormal clone for a minimum total of 2 karyotypes.
   - For cases with all normal cells:
     - Two karyotypes.

II. Patients who have undergone an allogeneic hematopoietic cell transplantation for whom donor vs recipient origin of the cells can be determined:

For studies aimed solely at determining engraftment status, molecular methods using different types of markers (eg, short tandem repeat, single-nucleotide polymorphism [SNP], and indel) are more analytically sensitive than

G-banded chromosome analysis and are the preferred methodologies.[20-22] Cytogenetic analyses should only be performed to exclude additional abnormalities or assess remission status. Therefore, in consultation with the referring physician, cancellation of test requests for G-banded chromosome analysis for engraftment status may be considered. Interphase FISH analysis using centromeric probes for the X and Y chromosomes (in the case of sex discordant transplant) can be used to determine the percentages of donor and recipient cells but may have limited sensitivity.

Cytogenetic analysis can uncover evidence of chimerism for recipient and donor cells based on sex chromosome complement in the case of sex discordant transplant and in the rare instance of a constitutional (ie, germline) structural chromosomal abnormality or an obvious chromosomal polymorphism in either the donor or recipient cells. It is expected that there will be different approaches used by different laboratories to address these studies based on the following scenarios:

- If only donor cells are present:
  a. Analysis: analyze 20 cells.
  b. Documentation: Document 2 karyotypes for each cell line. In such cases, one is documenting either the constitutional karyotype (normal or abnormal) of the donor or the rare event of a malignant process arising in a donor cell.
- If donor and recipient cells are present:
  a. Analysis: analyze recipient cells completely for previously identified clonal chromosome abnormalities and any newly acquired abnormalities. In some cases, there may be structural chromosomal abnormalities secondary to chromosome breakage or rearrangement induced by the pretransplant conditioning regimen. The laboratory should distinguish clonal from nonclonal changes and determine the clinical significance of newly detected abnormalities.
     1) Recipient cells: analyze all recipient cells present out of 20 cells analyzed. Evaluate each recipient cell for the presence of the abnormality present before transplantation (ie, the diagnostic abnormality). Depending on the number of recipient cells present among the initial 20 metaphase cells analyzed, additional recipient cells may be analyzed completely and/or scored for the presence of the diagnostic abnormality.
     2) Donor cells: analyze 2 donor cells if donor cells have not been analyzed in previous studies. Otherwise, simply score these cells as being of donor origin and count their modal number.
  b. Documentation:
     1) Recipient cells: 2 karyotypes for the stemline and 1 for each sideline.
     2) Donor cells: if donor cells have been documented previously, then provide a single metaphase spread. If donor cells have not been documented previously, then provide 2 karyotypes.
- If *only recipient cells* are present:
  a. Analysis: analyze 20 cells following the guidelines set forth above with respect to the characterization of the diagnostic, as well as secondary abnormalities.
  b. Documentation: same as noted above for abnormal recipient cells.

III. Patients who have undergone an allogeneic hematopoietic cell transplantation for whom donor and recipient cells cannot be determined: analyze 20 cells. As in the case scenarios outlined above, follow guidelines for recipient cells as set forth above.

6.5.2. FISH analysis

6.5.2.1 Interphase FISH analysis may be used as a primary testing methodology or in conjunction with G-banded chromosome analysis for the evaluation of hematologic neoplasms. FISH studies may be indicated to (i) provide a rapid result to aid in the differential diagnosis or therapy planning, (ii) detect a cryptic chromosomal abnormality or gene rearrangement, especially when G-banded chromosome analysis yields normal results, (iii) detect clinically significant gene amplification, which may also require metaphase FISH analysis to document the tandem nature of this rearrangement on the same chromosome (ie, signal clustering within the same chromosome vs copy-number gain on separate chromosomes), (iv) provide an alternative diagnostic method when no metaphase cells are obtained by blood, bone marrow, or lymphoid tissue cultures, and (v) detect abnormalities in samples that are not adequate or not suitable for G-banded chromosome analysis.

6.5.2.2 Characterization of the initial diagnostic interphase FISH abnormal signal pattern is important and will allow future monitoring of the patient's disease.

6.5.2.3 Metaphase FISH analysis and/or sequential G-banded chromosome analysis with metaphase FISH follow-up provides a useful methodology to characterize cryptic or variant chromosomal abnormalities or gene rearrangements (eg, gene fusion because of a 3-way translocation or an insertion).

6.5.2.4 Sample types that can be used for FISH analysis include (a) direct harvest or cultured fixed cells, (b) aspirate smears, (c) touch imprint preparations, (d) cytospin preparations, or (e) FFPE tissue sections.

a) Direct harvest or cultured fixed cells: These are the most commonly used preparations in hematologic malignancies and have multiple applications for both interphase and metaphase FISH analysis as discussed above.
b) Aspirate smears: hybridization of probes should be limited to areas of optimal cell density (cellular trails or the feathered edge of smears, without probing the spicules).

c) Touch imprint preparations: a pathologist should be involved in selecting the tissue for touch imprint preparations. These preparations should be made by lightly touching the piece of tumor tissue to a glass slide without smearing, followed by air drying.
d) Cytospin preparations: these are useful for a concentration of samples with very low cellularity (eg, cerebrospinal fluid).
e) FFPE tissue sections[23]:
   - Tumor sections cut to a validated thickness and mounted on positively charged organosilane-coated (silanized) slides work well. The cytogenetics laboratory should request several unstained sections and one hematoxylin and eosin–stained sequentially cut section from the submitting laboratory.
   - Before scoring a FFPE FISH slide, it is crucial for a pathologist to review a hematoxylin and eosin–stained slide and delineate the region of tumor cells that should be scored because it can be difficult to differentiate normal cells from malignant cells using only 4',6-diamidino-2-phenylindole (DAPI) counterstain. Before scoring the slide, the technologist should be clear where the malignant cells of interest are located on the slide.

6.5.2.5 A good understanding of the design of all FISH probe mixtures, especially dual-fusion and break-apart FISH probes, is critical for the accurate interpretation of FISH results. For dual-fusion probes, the level of coverage of the genes and flanking genomic regions should be taken into consideration when interpreting variant abnormal signal patterns. For break-apart probes, a clear distinction should be made between probes that span the 5′ and 3′ regions of the gene vs probes that span the flanking regions, and this distinction should be correlated with rearrangements that result in gene fusion (eg, *KMT2A* gene rearrangement) vs gene dysregulation (eg, *MYC* gene rearrangement). A variant abnormal interphase signal pattern should be interpreted in the context of the hematopathology findings, G-banded chromosome analysis, abnormal cell percentage (ie, abnormality involving the stemline or sideline clones), and whether the 5′ or 3′ region of the gene is the functionally significant portion of the gene fusion/rearrangement (eg, deletion of 3′ *KMT2A* with retention of the 5′ region, and involving the stemline clone is often interpreted as *KMT2A* gene rearrangement). Metaphase FISH analysis is often required to characterize variant chromosomal rearrangements.

6.5.2.6 Analysis and documentation of FISH studies should be in accordance with Section E9 of these technical standards for clinical genetics laboratories.

6.5.3. CMA analysis

6.5.3.1 CMA analysis can add valuable information that will support and supplement both G-banded chromosome and FISH analyses. It can detect small cryptic and clinically significant copy-number abnormalities (CNAs) in various hematologic malignancies. In addition, CMA SNP platforms can also detect copy-neutral loss of heterozygosity (CN-LOH). This technology, however, cannot detect balanced chromosomal rearrangements. Published clinically applicable data now show the clinical utility of CMA in the assessment of various hematologic malignancies.[13,24-27] Examples of the clinical utility of CMA in hematologic malignancies can be found in the disease-specific section discussed below (Section 6.5.4) and the ACMG/CGC technical laboratory standards for interpretation and reporting of acquired CNAs and CN-LOH in neoplastic disorders.[28]

6.5.3.2 In hematologic malignancies, CMA analysis is often performed on fresh bone marrow or peripheral blood for neoplastic studies but can also be performed on FFPE tissue. It is currently established as an accepted adjunct test for the characterization of cytogenetic abnormalities, especially in known disease entities where the common tumor driver has not been detected (eg, high-grade B cell lymphomas with 11q aberrations).[4,6]

6.5.3.3 Analysis and documentation of CMA studies should be in accordance with Section E10 of these technical standards for clinical genetics laboratories.

6.5.4. Recommended cytogenetic analysis scheme in hematologic neoplasms

6.5.4.1 Acute leukemias

Bone marrow is the preferred specimen for acute leukemias, but peripheral blood can be used when the percentage of circulating neoplastic cells exceeds the analytical sensitivity of the assay. Interphase FISH analysis performed on bone marrow smears or core biopsy touch imprint preparations is an alternative in cases with a dry tap and/or hemodiluted bone marrow aspirate and absent/low circulating blast cells. A close collaboration with the oncologist and pathologist is recommended for establishing the order of testing and additional tests that should be undertaken.

1. AML
   - G-banded chromosome analysis is indicated for all AML cases at diagnosis and relapse and is typically sufficient to identify cytogenetic abnormalities in AML clones.[29,30] However, some laboratories choose to combine FISH probes for the most common and clinically significant abnormalities into an AML FISH panel, which is performed on diagnostic specimens concurrently with the G-banded chromosome studies. This facilitates achieving clinically appropriate TAT for detection of key abnormalities. FISH confirmation is also useful in cases with poor chromosome morphology, and establishment of the FISH pattern at diagnosis may have utility for future follow-up.
   - Laboratories that offer AML FISH panel testing may consider including the following probes (Figure 2) [2,3,5,31,32]:
     a) *RUNX1::RUNX1T1* fusion probes for the t(8;21)(q22;q22)

Acute Leukemias			**Myelodysplastic neoplasms (MDS)**	**Myeloproliferative neoplasms (MPN) and myelodysplastic/myeloproliferative neoplasms**		**Myeloid/lymphoid neoplasms with eosinophilia**	**Plasma cell neoplasms (PCN)**	**Chronic lymphocytic leukemia (CLL)**
Acute Myeloid Leukemia (AML)	**Acute Lymphoblastic Leukemia (ALL)**			**Chronic Myeloid Leukemia (CML)**	**Other MPNs and MDS/MPN**			
	B-ALL	**T-ALL**						
RUNX1::RUNX1T1 fusion probes for the t(8;21)(q22;q22)	**For pediatric/young adult cases:**	*BCR::ABL1* fusion probes: for *BCR::ABL1* fusion and *ABL1* amplification	-5/5q- probes	*BCR::ABL1* fusion probes	*BCR::ABL1* fusion probes (for exclusion of CML)	*FIP1L1::PDFGRA* fusion probes (*CHIC2* deletion)	*IGH* rearrangement probes	*ATM* (11q22.3) probe
CBFB rearrangement or *CBFB::MYH11* fusion probes for the inv(16)(p13.1q22) and t(16;16)(p13.1;q22)	*BCR::ABL1* fusion probes	*KMT2A (MLL)* rearrangement probes	-7/7q- probes			*PDGFRB* rearrangement probes	1q21.3 probe (including *CKS1B*) for 1q21 copy gain and 1p32.3 probe (including *CDKN2C*) for 1p32 deletion	Centromeric probe for chromosome 12
KMT2A (MLL) rearrangement probes	*KMT2A (MLL)* rearrangement probes	*TCRA/D* rearrangement probes	Centromeric probe for chromosome 8			*FGFR1* rearrangement probes	*TP53* (17p13.1) probe	13q14.3 (including *D13S319*) probe
-5/5q- probes	*ETV6::RUNX1* fusion probes: for *ETV6::RUNX1* fusion, *ETV6* deletion, and iAMP21	**Optional:**	*TP53* (17p13.1) probe			*JAK2* rearrangement probes	Probes for three or more of the odd-numbered chromosomes (eg, chromosomes 5, 7, 9, 11, 15, and 19)	*TP53* (17p13.1) probe
-7/7q- probes	Centromeric probes for chromosomes 4 and 10	*BCL11B* rearrangement probes (particularly in ETP-ALL)	20q- probe			*ETV6::ABL1* fusion probes	13q14.2q14.3 probes (including *RB1*)	**Optional:**
TP53 (17p13.1) probe	**For adult cases:**						**Optional:**	*IGH::CCND1* fusion probes (in cases with atypical immunophenotype)
NUP98 rearrangement probes (in pediatric AML)	*BCR::ABL1* fusion probes						*MYC* rearrangement probes	
Depending on cell morphology, flow cytometry, G-banded chromosome analysis, and molecular studies:	*KMT2A (MLL)* rearrangement probes							
PML::RARA fusion probes for the t(15;17)(q24.1;q21.2)	**In both pediatric and adult B-ALL, if initial G-banded chromosome analysis and FISH panel testing is negative:**							
BCR::ABL1 fusion probes for the t(9;22)(q34.1;q11.2)	*CRLF2* rearrangement probes							
DEK::NUP214 fusion probes for the t(6;9)(p22.3;q34.1)	*PDGFRB* rearrangement probes							
MECOM (EVI1) rearrangement probes	*ABL1* rearrangement probes							
MLLT10 rearrangement probes or *KMT2A::MLLT10* fusion probes for the t(10;11)(p12;q23.1)	*ABL2* rearrangement probes							
	JAK2 rearrangement probes							

FISH probes recommended in a panel

Individual FISH probe recommendation

Figure 2 FISH panels and probes recommended in hematologic malignancies.

b) *CBFB* rearrangement or *CBFB::MYH11* fusion probes for the inv(16)(p13.1q22) and t(16;16)(p13.1;q22). FISH confirmation is advised for cases with inv(16) and t(16;16) because these abnormalities can be subtle, in particular if the morphology of G-banded chromosomes is suboptimal.
c) *KMT2A* (*MLL*) rearrangement probes
d) -5/5q- probes
e) -7/7q- probes
f) *TP53* (17p13.1) probe: for *TP53* deletion

- The primary FISH panel may also include *NUP98* rearrangement probes; in particular, for pediatric cases in which *NUP98* abnormalities are more common (Figure 2). *NUP98* rearrangements define specific entities in the WHO2022 (AML with *NUP98* rearrangement) and ICC2022 (AML with other rare recurring translocations) classifications, are often cryptic,[33,34] and their presence is associated with an unfavorable outcome.[35]
- Depending on cell morphology, flow cytometry, and/or results of G-banded chromosome analysis and molecular genetic testing, the following FISH probes can be added (Figure 2):
  - *PML::RARA* fusion probes for the t(15;17)(q24.1;q21.2). *PML::RARA* fusion is diagnostic of acute promyelocytic leukemia, which is usually strongly suspected at diagnosis based on the patient's presentation, blast cell morphology, and flow results. If acute promyelocytic leukemia is suspected, FISH for *PML::RARA* may be initiated at the same time as G-banded chromosome analysis in an expedited manner. A *RARA* break-apart probe can be used to detect rare variant translocations in which *RARA* fuses with a different partner gene.
  - *BCR::ABL1* fusion probes for the t(9;22)(q34.1;q11.2). AML with t(9;22) is rare but represents a diagnostic entity in the WHO2022 and ICC2022 classifications.
  - *DEK::NUP214* fusion probes for the t(6;9)(p22.3;q34.1). The t(6;9) can be subtle if the quality of G-banded chromosomes is poor. Additionally, it defines a specific entity in the WHO2022 and ICC2022 classifications and has prognostic significance.
  - *MECOM (EVI1)* rearrangement probes should be considered when chromosome analysis is suggestive of an inv(3)(q21q26.2), t(3;3)(q21;q26.2) or any abnormality involving 3q26.2. Rearrangements involving the *MECOM (EVI1)* locus at 3q26.2 define an entity in the WHO2022 and ICC2022 classifications and are associated with a very poor prognosis.
  - *MLLT10* rearrangement probes or *KMT2A::MLLT10* fusion probes for the t(10;11)(p12;q23.1). *MLLT10* translocations can be difficult to identify by G-banded chromosome analysis because they are frequently cryptic or associated with a complex karyotype.[36] For example, the t(10;11)(p12;q23.1) resulting in the *KMT2A::MLLT10* fusion has been reported to be cryptic in about 26% of cases, and can also result in a normal *KMT2A* break-apart probe FISH pattern.[37] FISH testing for an *MLLT10* rearrangement or specifically for the *KMT2A::MLLT10* fusion (which is the most common *MLLT10* fusion in AML) can be considered if results from other testing modalities are uninformative.
- Several other rare gene fusions (including *NPM1::MLF1, KAT6A::CREBBP, ETV6::MNX1, FUS::ERG, CBFA2T3::GLIS2* and others) define the AML with "other defined genetic alterations" or "rare recurring translocations" entities in the WHO2022 and ICC2022 classifications, respectively. Offering clinically validated FISH assays for all diagnostic rare fusions in AML may not be feasible for most laboratories; the choice of additional FISH probes to include in the testing menu may depend on clinical needs, patient population, accessible resources, and available molecular fusion testing at each institution.
- CMA testing in AML has been shown to detect abnormalities that influence risk stratification and patient management, including abnormalities undetectable by other routinely used testing modalities.[24] Assuming successful G-banded chromosome analysis, CMA testing may not be clinically indicated for every newly diagnosed AML patient. However, clinical use of CMA testing should be considered in the following circumstances: (1) normal karyotype, non-specific cytogenetic abnormalities and chromosome abnormalities associated with intermediate prognosis, (2) completely unobtainable or inadequate (fewer than 20 apparently normal analyzable metaphase cells) results by G-banded chromosome analysis, (3) unusual morphologic, immunophenotypic or cytogenetic findings, and (4) refractory and relapsed AML.[24] In addition, CMA with a SNP component is the most reliable testing modality for detection of CN-LOH, which has been shown to have prognostic significance in AML and to "unmask" variants in oncogenes or tumor suppressor genes (eg, 13q CN-LOH with *FLT3* activating variants and 17p CN-LOH with *TP53* loss-of-function variants).[38,39]

2. ALL
   - B-ALL is more frequent than T-lineage ALL (T-ALL), accounting for 85% of pediatric ALL and 75% of adult ALL.[2,4,5]
   - In both pediatric/young adult and adult B-ALL, G-banded chromosome analysis should be performed simultaneously with interphase FISH analysis for

the most frequent clinically significant abnormalities in each age group.

- In pediatric B-ALL, it is recommended to include the following probes in the initial FISH panel (Figure 2):
  a) *BCR::ABL1* fusion probes
  b) *KMT2A* (*MLL*) rearrangement probes
  c) *ETV6::RUNX1* fusion probes: for *ETV6::RUNX1* fusion, *ETV6* deletion, and iAMP21 (intrachromosomal amplification of chromosome 21)
  d) Centromeric probes for chromosomes 4 and 10 for trisomies of chromosomes 4 and 10
- In adult B-ALL, the following probes are recommended for the initial interphase FISH analysis (Figure 2):
  a) *BCR::ABL1* fusion probes
  b) *KMT2A (MLL)* rearrangement probes
- When feasible, *BCR::ABL1* FISH analysis of flow-sorted cells or in combination with cell morphology (segmented vs mononuclear cells) may be important in distinguishing between CML in lymphoid blast phase and de novo B-ALL.[5]
- If the initial G-banded chromosome analysis and FISH panel testing does not identify any diagnostic genetic abnormalities, in both pediatric and adult B-ALL, additional FISH testing is recommended for abnormalities associated with the *BCR::ABL1*-like (Philadelphia [Ph]-like) disease subtype. The following FISH probes are recommended, targeting the most frequent abnormalities in Ph-like B-ALL (Figure 2):
  a) *CRLF2* rearrangement probes: if positive for the typical disruption pattern, then there is likely an *IGH::CRLF2* fusion due to the t(X/Y;14)(p22.33/p11.32;q32.33) rearrangement. However, if positive for a signal pattern suggestive of the pseudoautosomal region 1 (PAR1) deletion, follow-up FISH testing is recommended using the *P2RY8* and *IGH* break-apart probes to confirm the *P2RY8::CRLF2* fusion and exclude a concomitant *IGH::CRFL2* fusion. Metaphase FISH testing using the *CRLF2* and *IGH* break-apart probes or interphase FISH testing using the *IGH::CRLF2* dual-fusion probes may be needed. The concomitant presence of the t(X/Y;14) and PAR1 deletion involving the same *CRLF2* allele functionally behaves similar to the *IGH::CRLF2* fusion because the translocation breakpoint maps proximal to the *CRLF2* gene and the whole gene is translocated to the der(14) in close proximity to the *IGH* enhancer.[40]
  b) *PDGFRB* rearrangement probes
  c) *ABL1* rearrangement probes
  d) *ABL2* rearrangement probes
  e) *JAK2* rearrangement probes
- The *ABL1, ABL2, JAK2,* and *PDGFRB* genes constitute the 3′ gene fusion partner in Ph-like B-ALL, and a break-apart probe signal pattern demonstrating a deletion of the 5′ end of the gene is considered positive for a gene rearrangement.
- If the genetic driver remains unknown after evaluation for Ph-like B-ALL, additional interphase FISH testing may be considered to detect abnormalities that define other specific entities in the WHO2022 and ICC2022 classifications, have prognostic and/or predictive significance, or are frequently observed in B-ALL. Further FISH testing may also be guided by the patient's clinical characteristics (age of onset, constitutional trisomy 21, residual disease) or flow cytometry findings. Clinical laboratories increasingly rely on molecular next-generation sequencing (NGS) based testing for detection of abnormalities associated with novel subtypes of B-ALL, as some of the relevant FISH probes may not be commercially available. If additional testing by FISH is pursued, the following probes may be selected:
  a) *TCF3::PBX1* fusion probes
  b) *TCF3::HLF* fusion probes
  c) *ZNF384* rearrangement probes
  d) *MEF2D* rearrangement probes
  e) *NUTM1* rearrangement probes
  f) *IGH::IL3* fusion probes
  g) *MYC* rearrangement and/or *IGH::MYC* fusion probes
  h) *PAX5* (9p13.2) probe
  i) *CDKN2A/B* (9p21.3) probe: 9p21.3 deletion is common in both B- and T-ALLs, but its prognostic significance has been debated; however, it provides a clonal target for future monitoring of the patient's disease in the absence of other FISH targets.
- In T-ALL, G-banded chromosome analysis should be performed first. Interphase FISH analysis is optional and could include the following probes (Figure 2):
  a) *BCR::ABL1* fusion probes: for *BCR::ABL1* fusion and *ABL1* amplification (ie, episomal amplification of the *NUP214::ABL1* fusion)
  b) *KMT2A (MLL)* rearrangement probes
  c) *TCRA/D* rearrangement probes
- FISH for *BCL11B* rearrangement may be considered, particularly in the diagnosis of early T cell precursor ALL (ETP-ALL) (Figure 2). Approximately a third of ETP-ALL is characterized by rearrangement and deregulation of *BCL11B*. *BCL11B* rearrangements now define a specific entity in the ICC2022 classification (ETP-ALL with *BCL11B* rearrangement) and are often cryptic.[41]
- CMA, when combined with G-banded chromosome and FISH analyses, can significantly enhance the genetic profiling of both B-ALL and T-ALL.[27,42-45] In B-ALL, CMA can provide evidence for *IKZF1* deletion, including the newly defined *IKZF1*-plus entity,[46] *ERG* deletion (present in 50% of cases with *DUX4*

rearrangement),[47] and the diagnostic pattern of chromosome 21 CNAs associated with iAMP21, which may be missed by *RUNX1* FISH analysis alone.[48] A SNP CMA is also very helpful in distinguishing the favorable hyperdiploid B-ALL from the unfavorable near haploid or low hypodiploid B-ALL that often doubles and presents in the form of hyperdiploidy or near triploidy. In T-ALL, CMA detects cryptic deletions at 1p32 that result in *STIL::TAL1* fusion and 9q34 amplification because of episomal amplification of the *NUP214::ABL1* fusion.[49,50]

6.5.4.2 Myelodysplastic neoplasms or syndromes

- Bone marrow is the preferred specimen for MDS.[51] Interphase FISH analysis performed on bone marrow smears or core biopsy touch imprints is an alternative in cases with a dry tap and/or hemodiluted bone marrow aspirate. A close collaboration with the oncologist and pathologist is recommended in MDS cases because other non-neoplastic hematologic disorders can have a similar presentation.
- G-banded chromosome analysis is recommended to be performed first.[52] In case of an incomplete/unsuccessful chromosome analysis or if the laboratory is unable to maintain a clinically appropriate TAT for chromosome analysis, CMA analysis or MDS FISH panel should be performed on the diagnostic specimen (NCCN MDS Guidelines).[53]
  - The MDS FISH panel may include the following set of probes that target common abnormalities (Figure 2):[53]
    a) -5/5q- probes
    b) -7/7q- probes
    c) Centromeric probe for chromosome 8: for trisomy 8
    d) *TP53* (17p13.1) probe: for *TP53* deletion in conjunction with somatic variant testing to identify multi-hit *TP53* lesions which define their own category of MDS with biallelic *TP53* inactivation in WHO2022 or MDS with mutated *TP53* in ICC2022.[3,5,54]
    e) 20q probe: for 20q deletions
  - The Revised International Prognostic Scoring System (IPSS-R) also includes -Y, 11q-, 12p-, +19, and inv(3)/t(3;3) based on karyotypic findings. In the case of a suggestive but inadequate karyotype, laboratories may consider confirmatory testing for these abnormalities by FISH analysis.[53,55]
- The WHO and ICC classifications of myeloid neoplasms indicates that the presence of trisomy 8, 20q deletion, or -Y is not considered to be MDS defining in the absence of morphologic features of MDS.[3,5] However, loss of the Y chromosome (LOY) in ≥75% of bone marrow cells has been reported to be associated with an increased likelihood of molecular aberrations in genes commonly seen to be altered in myeloid neoplasia and with morphological features of MDS. This argues that ≥75% LOY in bone marrow may be considered an MDS-associated cytogenetic abnormality.[55] Some laboratories may opt to use trisomy 8 and 20q deletion probes for detecting and monitoring the abnormal clone because these abnormalities (in particular trisomy 8) are relatively common. In addition, these abnormalities are no longer defining for AML, myelodysplasia related.[3,5]
- CMA has been shown to be an extremely useful diagnostic tool for the workup of patients with MDS, *BCR::ABL1* fusion-negative MPN, and MDS/MPN, along with chromosome analysis, FISH, and variant analysis. In these myeloid neoplastic disorders, clonal CNAs and CN-LOH are the most common chromosomal abnormalities, whereas balanced structural abnormalities do not play a major role.[25] CN-LOH spanning *TP53* in conjunction with a *TP53* variant would fulfill diagnostic criteria in the correct pathologic setting for MDS with biallelic *TP53* inactivation or MDS with mutated *TP53*.[3,5] In MDS, CMA is recommended in case of an incomplete or unsuccessful G-banded chromosome analysis, as well as in patients with a normal karyotype or with uncertain IPSS-R cytogenetic risk-scores to achieve accurate risk stratification. CMA can uncover clinically significant clonal CNAs and/or CN-LOH in these situations.[25]
- Baseline FISH analysis for clonal stemline abnormalities (based on chromosome analysis and CMA findings) should always be considered to identify an informative probe (previously validated in the laboratory) for future monitoring of measurable residual disease.
- Establishing a diagnosis of MDS is often challenging in the absence of clear evidence for morphologic dysplastic changes or MDS-specific cytogenetic abnormalities. A large amount of data has become available on recurring somatic clonal SNVs in MDS, and the identification of a variant in *SF3B1* now defines a subcategory of MDS, in addition to the categories associated specifically with *TP53* lesions.[3,5] NGS can detect variants in 90% of MDS patients.[2,56] However, clonal hematopoiesis of indeterminate potential (CHIP) has been defined as somatic clonal SNVs in myeloid neoplasm driver genes (also recurrently mutated in MDS) detected in the blood or bone marrow at a variant allele fraction of ≥2% in patients lacking a myeloid neoplasm or unexplained cytopenia.[3,5] Thus, the presence of MDS-associated somatic clonal SNVs alone is not considered diagnostic of MDS in the WHO classification of myeloid neoplasms.[3] In addition, clonal CN-LOH spanning known myeloid neoplasm-associated genes can also reveal clonal hematopoiesis and warrants a more rigorous follow-up schedule for these patients aimed at the early detection of a myelodysplastic disease.[57]

6.5.4.3 Myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms

This is a heterogeneous group of clonal stem cell disorders that is broadly divided into 2 groups.[3,5] The first is the MPN

group, which includes CML, polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, chronic eosinophilic leukemia, juvenile myelomonocytic leukemia (WHO2022), and MPN not otherwise specified (also called MPN unclassifiable in ICC2022). The second group is the MDS/MPN group, which includes chronic myelomonocytic leukemia, clonal cytopenia with monocytosis of undetermined significance (ICC2022), clonal monocytosis of undetermined significance (ICC2022), MDS/MPN with neutrophilia (also called atypical CML in ICC2022), MDS/MPN with *SF3B1* variant and thrombocytosis, MDS/MPN with ring sideroblasts and thrombocytosis not otherwise specified (ICC2022), and MDS/MPN not otherwise specified (also called MDS/MPN unclassifiable in ICC2022).

1. CML
   - Bone marrow or peripheral blood is adequate to establish the diagnosis of CML. However, bone marrow is required at the time of diagnosis to assess for accelerated phase (ICC2022, or high-risk chronic phase in WHO2022) or blast phase disease that might not be present in the peripheral blood. Therefore, G-banded chromosome analysis on the bone marrow is recommended in situations where the bone marrow specimen is available.
   - The t(9;22)(q34;q11.2) is detectable in 90% to 95% of CML cases at diagnosis. The remaining 5% to 10% of cases have either a variant t(9;22) or a cryptic *BCR::ABL1* fusion undetectable by G-banded chromosome analysis.
   - The NCCN CML Guidelines recommend that both G-banded chromosome analysis and quantitative RT-PCR for *BCR::ABL1* fusion testing be performed at diagnosis. If no *BCR::ABL1* fusion can be detected, molecular testing for variants associated with other myeloproliferative conditions is indicated (NCCN CML Guidelines).
   - It is important to assess whether additional chromosome abnormalities are present at diagnosis, including an additional der(22)t(9;22), trisomy 8, i(17q), trisomy 19, complex karyotype, and abnormalities of 3q26.2. The presence of any one of these abnormalities is indicative of progressive disease.[2,5,58,59]
   - FISH for *BCR::ABL1* fusion can be performed if G-banded chromosome analysis is not possible and for monitoring atypical *BCR* breakpoints resulting in the inability to detect the fusion by RT-PCR (NCCN CML Guidelines) (Figure 2).
2. Other MPNs and MDS/MPNs
   - Bone marrow is the preferred specimen for other MPNs; however, peripheral blood may be used if there is peripheral involvement. With few exceptions, cytogenetic abnormalities are usually not specific in other MPNs or MDS/MPNs. Typical abnormalities of myeloid neoplasms are usually observed and can be useful in demonstrating evidence of clonality.
   - G-banded chromosome analysis should preferably be performed first.
   - The exclusion of *BCR::ABL1* fusion is required for the diagnosis of other MPNs and MDS/MPNs from CML (NCCN MPN Guidelines).[3,5]
   - Interphase FISH analysis performed on bone marrow smears or core biopsy touch imprints is an alternative in cases with a dry tap and/or hemodiluted bone marrow aspirate. A close collaboration with the oncologist and pathologist is important for the choice of FISH probes but the workup should include the exclusion of the *BCR::ABL1* fusion (Figure 2).
   - In *BCR::ABL1* fusion-negative MPNs, CMA may be helpful as a reflex to normal or failed chromosome analysis.[25]
   - Molecular testing for variants associated with other myeloproliferative conditions is required for the diagnosis of MPNs and MDS/MPNs (NCCN MPN Guidelines).[3,5]

6.5.4.4 Myeloid/lymphoid neoplasms with eosinophilia

- Bone marrow is the preferred specimen for myeloid/lymphoid neoplasms with eosinophilia; however, peripheral blood may be used if there is involvement of the latter.
- G-banded chromosome analysis and FISH should both be performed in cases with high pathologic and clinical suspicion for one of these entities. Targeted NGS technologies aimed at detection of gene fusions may be considered as well.
- Specific FISH probes that detect *FIP1L1::PDFGRA* fusion (cryptic by chromosome analysis and typically detected by evaluation for *CHIC2* deletion by FISH), *PDGFRB* rearrangement, *FGFR1* rearrangement, *JAK2* rearrangement, and *ETV6::ABL1* fusion are recommended (NCCN Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Fusion Genes Guidelines) (Figure 2).[3,5] Cases with these gene rearrangements may be targetable by specific therapies (ie, tyrosine kinase inhibitors).[3,5,60-64]
- Rarely, other kinase gene fusions have been identified that appear to behave similarly to the more common kinase fusions and may be targetable as well (eg, *FLT3, FGFR2,* and *LYN*). Where probes are available and validated by the laboratory, confirmatory FISH studies are recommended (NCCN Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Fusion Genes Guidelines).[65-68]

6.5.4.5 PCNs

- A bone marrow specimen is required for PCNs. For FISH and/or CMA analyses, CD138+ enriched samples are

strongly recommended for optimized yield (see Section 6.4.2.3 for optimal processing of bone marrow specimens for PCN workup) (NCCN MM Guidelines).[11,12]

- G-banded chromosome analysis may be performed (as described above). However, unless it is utilized to demonstrate a complex karyotype and thus inform the aggressive nature of the dividing plasma cells, it is not required for risk stratification.[69] If there is a concern for another hematologic malignancy, such as a myeloid neoplasm, a chromosome analysis may be warranted.
- Appropriate risk stratification is best achieved using the following panel of FISH probes in the following order of priority (Figure 2):
  a) *IGH* rearrangement probes: if *IGH* is rearranged, including the classical gene disruption, as well as deletion of either the 5′ or 3′ region of *IGH*, then reflex to the following dual-fusion probes: *IGH::FGFR3* and *IGH::NSD2* [t(4;14)(p16;q32)], *IGH::CCND1* [t(11;14)(q13;q32)], *IGH::MAF* [t(14;16)(q32;q23)], and *IGH::MAFB* [t(14;20)(q32;q12)], with optional inclusion of *IGH::CCND3* [t(6;14)(p21;q32)].[4,6]
  b) 1q21.3 probe (including the *CKS1B* gene region) for 1q21 copy gain and 1p32.3 probe (including the *CDKN2C* gene region) for 1p32 deletion, both of which have been linked to adverse prognosis (NCCN MM Guidelines).[70,71]
  c) *TP53* (17p13.1) probe: monoallelic deletions may need to be followed with *TP53* sequencing as biallelic alterations are associated with poor outcome (NCCN MM Guidelines).[72]
  d) Probes for 3 or more of the odd-numbered chromosomes that are often trisomic in hyperdiploid PCN (eg, chromosomes 5, 7, 9, 11, 15, and 19). These probes can also detect hyperhaploidy, characterized by odd-numbered chromosomes being disomic and the other chromosomes being monosomic,[73] a PCN entity that has been reported to be associated with high-risk abnormalities (eg, *TP53* variant) and a poor prognosis.[74-76]
  e) 13q14.2q14.3 probes (including *RB1*): 13q14.2q14.3 deletion is common in PCN, but when detected only by FISH, is not predictive of survival in the absence of other adverse chromosomal abnormalities. It is worth noting that 13q deletion detected by G-banded chromosome analysis still retains its prognostic value (NCCN MM Guidelines).
- Of note, laboratories may choose to include a *MYC* probe to the above panel, as *MYC* rearrangements are associated with high disease burden and represent an independent adverse factor in patients with newly diagnosed PCN (Figure 2).[4,6,77] Furthermore, *MYC* rearrangements are present in 40% of PCN and can contribute to the progression of MM.[78]
- The use of CMA analysis, particularly in combination with FISH, on the enriched plasma cell fraction has been shown to be valuable in detecting clinically relevant CNAs and can be used to detect chromothripsis.[79-82] The increase in genomic instability leading to chromothripsis is a common feature of PCN and its detection using CMA may inform more accurate risk predictions.[83] In addition to hyperdiploidy of odd-numbered chromosomes and gain of 1q, several numerical aberrations involving other chromosomes have been described in PCN.[13]

6.5.4.6 CLL

- CLL is a mature B cell neoplasm diagnosed by abnormalities in B cell count, morphology, and flow cytometry. The evaluation of del(11q), del(13q), del(17p), trisomy 12, and *TP53* and *IGHV* variant status are essential for the prognosis prediction at the time of diagnosis of CLL.[4] Cytogenetically, either peripheral blood or bone marrow can be used for the workup of this disease. G-banded chromosome analysis using both unstimulated and CpG-oligonucleotides stimulated cultures should be performed simultaneously with interphase FISH analysis.[84] CpG-oligonucleotides stimulation greatly improves the detection rate of clonal cytogenetic abnormalities by G-banded chromosome analysis.[18,19] IL-2 or a combination of other conventional B cell stimulants may be added for optimal results.[18,19,85,86] A complex karyotype with ≥3 unrelated chromosomal abnormalities in CpG stimulated culture is a strong predictor of poor clinical outcome (NCCN CLL/SLL Guidelines); however, a complex karyotype with ≥5 unrelated chromosomal abnormalities may be a better predictor for the stratification of very high-risk patients.[6] G-banded chromosome analysis using unstimulated culture allows for the detection of independent clonal abnormalities in cases with concurrent hematologic malignancies, for example, de novo or therapy-related MDS.
- To assign the patient into clinically relevant prognostic subgroups, the following panel of FISH probes is recommended (NCCN CLL/SLL Guidelines) (Figure 2):
  a) *ATM* (11q22.3) probe
  b) Centromeric probe for chromosome 12 for trisomy 12
  c) 13q14.3 (including D13S319) probe
  d) *TP53* (17p13.1) probe
- In addition, FISH using the *IGH::CCND1* fusion probes may be considered in all cases, especially those with an atypical immunophenotype (ie, CD23 dim or negative, CD20 bright, surface immunoglobulin bright) to differentiate them from mantle cell lymphoma (MCL) (NCCN CLL/SLL Guidelines) (Figure 2).
- In CLL, CMA analysis has proven to be effective in detecting CNAs and CN-LOH of genomic regions with established prognostic significance and provides a much higher resolution compared with G-banded chromosome and FISH analyses.[26,87,88] Moreover, clinically relevant genomic alterations in CLL involve

Table 1 The typical cytogenetic findings in key types of lymphomas

Tumor	Chromosomal Aberrations	Clinical Significance	Reference, Publication Year
B CELL			
Burkitt lymphoma	t(8;14)(q24;q32) [*IGH::MYC*] t(2;8)(p12;q24) [*IGK*::*MYC*] t(8;22)(q24;q11.2) [*IGL::MYC*]	Characteristic *MYC* overexpression, and variant translocations	Saleh et al,[89] 2020
High grade B cell lymphoma with 11q aberrations (WHO2022)/Large B cell lymphoma with 11q aberration (ICC2022)	Complex aberrations of 11q with a minimal gain of 11q23.3 and minimal loss of 11q24.1qter	Identification of these disease-defining aberrations requires CMA analysis	Salaverria et al,[90] 2014
Diffuse large B cell lymphoma and high-grade B cell lymphoma with *MYC* and *BCL2* rearrangements	Gene rearrangements: 3q27 [*BCL6*], 8q24 [*MYC*], 18q21 [*BCL2*], 1p22 [*BCL10*]	High prevalence of *BCL6* rearrangements in this entity Unfavorable *MYC* translocations with *TP53* variants or as part of double-hit lymphomas with *BCL2* rearrangements	Rosenthal et al,[91] 2017 Slack et al,[92] 2011, Schuetz et al,[93] 2012, Zhou et al,[94] 2014
Follicular lymphoma, including pediatric-type follicular lymphoma and follicular large B cell lymphoma (WHO2022)/ Large B cell lymphoma with *IRF4* rearrangement (ICC2022)	t(14;18)(q32;q21) [*IGH::BCL2*] t(2;18)(p12;q21) [*IGK::BCL2*] t(18;22)(q21;q11.2) [*IGL::BCL2*] Other gene rearrangements: 3q27 [*BCL6*], 8q24 [*MYC*], 6p25 [*IRF4*] Other genomic findings: Loss or CN-LOH of 1p36 [*TNFRSF14*]	Characteristic *BCL2* overexpression, and variant translocations *IRF4* rearrangements and *TNFRSF14* loss or CN-LOH should be examined in BCL2-negative cases in the correct histologic and clinical setting	Mozas et al,[95] 2021 Bastard et al,[96] 1994, Bosga-Bouwer et al,[97] 2005, Louissaint et al,[98] 2016, Gángó et al,[99] 2018
Mantle cell lymphoma	t(11;14)(q13;q32) [*IGH::CCND1*] t(2;11)(p11;q13) [*IGK::CCND1*] t(11;22)(q13;q11) [*IGL::CCND1*] t(12;22)(p13;q21) [*IGL::CCND2*] t(2;12)(p12;p13) [*IGK::CCND2*] t(12;14)(p13;q32) [*IGH::CCND2*] t(6;14)(p21;q32) [*IGH::CCND3*] Other gene rearrangements: 8q24 [*MYC*], 3q27 [*BCL6*] Other genomic findings: Loss of 17p13 [*TP53*]	Characteristic *CCND1, CCND2,* or *CCND3* overexpression Complex karyotypes and loss of *TP53* associated with unfavorable prognosis	Michaux et al,[100] 2004, Gesk et al,[101] 2006, Wlodarska et al,[102] 2008, Navarro et al,[103] 2020
Splenic marginal zone lymphoma	Deletion 7q	del(7q) is the most common structural variant in this entity and is highly specific	Mateo et al,[104] 1999, Solé et al,[105] 2001, Salido et al,[106] 2010, Rinaldi et al,[107] 2011

(continued)

Table 1 Continued

Tumor	Chromosomal Aberrations	Clinical Significance	Reference, Publication Year
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	t(11;18)(q21;q21) [*BIRC3::MALT1*] t(14;18)(q32;q21) [*IGH::MALT1*] t(3;14)(p14;q32) [*IGH::FOXP1*] t(1;14)(p22;q32) [*IGH::BCL10]*	Characteristic overexpression of the immunoglobulin partner or *MALT1* associated with specific sites of disease and autoimmune or infectious etiologies	Maes et al,[108] 2000, Streubel et al,[109] 2003, Zhou et al,[110] 2006, Zhou et al,[111] 2007
ALK-positive large B cell lymphoma	t(2;17)(p23;q23) [*ALK::CLTC*] Other gene rearrangements: 2p23 [*ALK*, various partners]	Characteristic *ALK* overexpression	Stachurski et al,[112] 2007, Zhang et al,[113] 2009
T CELL			
ALK-positive, anaplastic large cell lymphoma (ALCL)	t(2;5)(p23;q35) [*NPM1::ALK*] Other gene rearrangements: 2p23 [*ALK*, various partners]	Characteristic *ALK* overexpression (cellular localization dependent upon the translocation partner)	Savage et al,[114] 2008, Schmitz et al,[115] 2010, Abate et al,[116] 2015
ALK-negative, ALCL	t(6;7)(p25.3;q32.2) [*DUSP22::FRA7H*] inv(3)(q26q28) [*TP63::TBL1XR1*] Other gene rearrangements: 19p13 [*VAV1, TYK2*], 6q22 [*ROS1*]	High prevalence in primary cutaneous ALCL; *DUSP22* rearrangements associated with good prognosis, whereas *TP63* with adverse prognosis	Feldman et al,[117] 2011, Vasmatzis et al,[118] 2012, Parrilla Castellar et al,[119] 2014, Crescenzo et al,[120] 2015, Boddicker et al,[121] 2016
Peripheral T cell lymphoma, not otherwise specified or Nodal T-follicular helper (TFH) cell lymphoma, angioimmunoblastic type (WHO2022)/Follicular helper T cell lymphoma, angioimmunoblastic type (ICC2022)	t(14;19)(q11;q13) [*TCRA/D* variants] t(5;9)(q33;q22) [*ITK::SYK*] 2q33.2 tandem duplication [*CTLA4::CD28* or *ICOS::CD28*]		Kataoka et al,[122] 2015, Yoo et al,[123] 2016
T-prolymphocytic leukemia (WHO2022)/T cell prolymphocytic leukemia (ICC2022)	inv(14)(q11;q32.1) [*TRA/D:: TCL1A/B*] t(14;14)(q11;q32.1) [*TRA/D:: TCL1A/B*] t(X;14)(q28;q11) [*TRA/D:: MTCP1*]	Characteristic *TCL1* overexpression Characteristic *MTCP1* overexpression	Staber et al,[124] 2019, Colon Ramos et al,[125] 2021
Hepatosplenic T cell lymphoma	iso(7q)	Characteristic finding in this diagnosis	Pro et al,[126] 2020

ALCL, Anaplastic large cell lymphoma; *CMA*, chromosomal microarray analysis; *TFH*, T-follicular helper.

mostly genomic gains and losses, with balanced rearrangements being less common and currently of uncertain prognostic value. DNA from fresh CLL samples is generally available, and the tumor burden tends to be relatively high in peripheral blood, which makes CLL particularly amenable to the detection of CNAs by CMA. In instances where FISH and CMA data are discrepant, CMA analysis can further refine deletion breakpoints and determine the clinical relevance of atypical deletions. CMA analysis can detect increased genomic complexity, which is an independent marker of aggressive CLL and poor outcome and can identify patients at risk for Richter transformation.[26]

6.5.4.7 B and T cell lymphomas

- G-banded chromosome analysis is recommended for all involved fresh tissues:
  - A preferred tissue is a lymph node or biopsy material from a suspected lymphoid mass.
  - Mitogen stimulation may be required for involved bone marrow or peripheral blood specimens of low-grade lymphomas.

- Interphase FISH analysis using relevant probes should be performed on lymphoid tissue sections, fine needle or bone marrow aspirate smears, and/or touch imprints. Metaphase FISH analysis can also be performed as needed.
- Lymphomas are a vast and diverse set of hematopoietic neoplasms, encompassing a wide range of cytogenetic aberrations. Although the lymphomas may harbor numerous chromosome rearrangements, including CNAs, many are non-specific. These cytogenetic alterations are usually identified only by CMA or G-banded chromosome analysis, methods that are infrequently applied at some institutions in the standard workup of lymphomas. For the typical cytogenetic findings in key types of lymphomas, please see Table 1. Of note, this table is not meant to be comprehensive, but it only serves to highlight the more commonly tested or diagnostically relevant translocations and CNAs. Wherever appropriate, a comparison between the WHO2022 and ICC2022 classifications was included in Table 1.[4,6]
  - The most common studies performed in B cell lymphomas are FISH evaluation for double and triple hit lymphomas, specifically for *MYC*, *BCL2*, and *BCL6* gene rearrangements. These probes can be performed as a panel or through reflex testing beginning with *MYC*. Some *MYC* rearrangements may be missed using only break-apart probes; therefore, additional testing using *IGH::MYC* dual-fusion probes may be indicated (see Section 6.5.2.5).[127]
  - For high-grade B cell lymphoma with 11q aberrations (WHO2022)/large B cell lymphoma with 11q aberration (ICC2022), the 11q aberrations are typically identified only by CMA.
  - NGS methods for either copy-number assessment or translocation identification (either RNA or DNA based) are not within the scope of this document. However, they can identify additional copy-number and structural alterations beyond those listed here.

6.6. TAT and reporting

6.6.1. TAT

6.6.1.1 Specific chromosomal abnormalities are crucial for establishing a diagnosis and have direct relevance to specific treatment. Therefore, an effort should be made to expedite communicating the cytogenetic results to the oncologist. It is recommended that the cytogenetics laboratory have a written policy describing how cases are prioritized.

6.6.1.2 TAT guidance:

a. Initial diagnostic workup: it is strongly recommended that the preliminary G-banded chromosome analysis result be reported within 7 calendar days or less, and the final result be reported within 21 calendar days.
b. Follow-up studies: It is strongly recommended that the final G-banded chromosome analysis result be reported within 21 calendar days.
c. FISH studies: Reporting the FISH results within 3 to 5 working days from the time of receiving the specimen is recommended whenever possible.
d. CMA studies: TAT should be optimized based on the clinical indication for CMA analysis and the hematologic neoplasm being studied.[13,24-27]

6.6.2. Reporting

6.6.2.1 The most recent edition of the International System for Human Cytogenomic Nomenclature (ISCN) should be used to report the cytogenetics results.[128]

6.6.2.2 The number of cells analyzed (both normal and abnormal) should be documented in the final report, when applicable.

6.6.2.3 For CMA analysis, clones and subclones cannot be ascertained with certainty; however, the percentage of cells (levels of mosaicism) can be provided (within the sensitivity limits of the microarray) to give an estimate of possible clones/subclones and clonal diversity.

6.6.2.4 If a potential non-mosaic constitutional abnormality is observed in oncology workups, analysis of a phytohemagglutinin-stimulated peripheral blood sample during remission is strongly recommended to confirm that the abnormality is constitutional and not clonal.

6.6.2.5 At the time of initial diagnosis, finding a single abnormal metaphase cell, even one that is potentially significant, cannot be used as evidence of clonality unless there is strong supporting evidence of clonality for the same abnormality by either FISH or other molecular technique.

6.6.2.6 The final cytogenetic report of hematologic acquired chromosomal abnormalities should contain the following information:

1. Patient identification using 2 different identifiers
2. Patient medical record number and/or laboratory identification number
3. Referring physician
4. Address of the testing institution
5. Sample information (type, date of withdrawal and receipt, and date of report)
6. Reason for referral or suspected diagnosis
7. ISCN nomenclature of cytogenetic studies performed
8. Narrative description of the abnormalities observed, including modal chromosome number in each clone (to the extent possible), and numerical and structural abnormalities. The report should comment on the clinical significance of the abnormalities observed, including clinically relevant genes involved, possible disease association, and prognostic significance.
9. When applicable, literature references to support the clinical interpretation and to provide helpful information for the referring physician.

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Conflict of Interest

All authors of these technical standards are directors of clinical laboratories that offer cytogenomic testing or hematopathology services to patients with hematologic malignancies. L.B.B. serves as a consultant for Genentech.

Affiliations

[1]The Steve and Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH; [2]Division of Hematopathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [3]Department of Pathology, University of Michigan, Ann Arbor, MI; [4]Department of Pathology, Baylor University Medical Center, Dallas, TX; [5]Texas A&M School of Medicine, Texas A&M University, Dallas, TX; [6]Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, CA; [7]Keck School of Medicine, University of Southern California, Los Angeles, CA; [8]Department of Genetics, University of Alabama at Birmingham, Birmingham, AL; [9]American College of Medical Genetics and Genomics, Bethesda, MD

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