# Protocol for the Examination of Specimens From Patients With Hematopoietic Neoplasms Involving the Bone Marrow*

**Version:** BoneMarrow 3.1.0.0 **Protocol Posting Date:** January 2018
**This protocol is NOT required for accreditation purposes**

*This protocol applies to bone marrow specimens involved by primary hematopoietic neoplasms. The plasma cell neoplasm protocol is recommended for reporting bone marrow samples with plasma cell myeloma.

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**Accreditation Requirements**
This protocol can be utilized for clinical care purposes, but is not required for accreditation purposes.

**CAP Laboratory Accreditation Program Protocol Required Use Date: Not applicable**

## CAP Bone Marrow Protocol Summary of Changes

**v3.1.0.0**
**The following data elements were modified:**
Histologic Type

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## Surgical Pathology Cancer Case Summary

Protocol posting date: January 2018

**BONE MARROW: Aspiration, Core (Trephine) Biopsy**

**Note: This case summary is recommended for reporting bone marrow specimens, but is not required for accreditation purposes.**

**Select a single response unless otherwise indicated.**

**Specimen (select all that apply) (Note A)**
___ Peripheral blood smear
___ Bone marrow aspiration
___ Bone marrow aspirate clot (cell block)
___ Bone marrow core (trephine) biopsy
___ Bone marrow core touch preparation (imprint)
___ Other (specify): ____________________________
___ Not specified

**Procedure (select all that apply)**
___ Aspiration
___ Biopsy
___ Other (specify): ____________________________
___ Not specified

**Aspiration Site (if performed) (select all that apply) (Note B)**
___ Right posterior iliac crest
___ Left posterior iliac crest
___ Sternum
___ Other (specify): ____________________________
___ Not specified

**Biopsy Site (if performed) (select all that apply) (Note B)**
___ Right posterior iliac crest
___ Left posterior iliac crest
___ Other (specify): ____________________________
___ Not specified

**Histologic Type (Note C)**
*Note: The following is a partial list of the 2017 World Health Organization (WHO) classification and includes those neoplasms seen in bone marrow specimens. The list does not include provisional entities.*

*[#]An initial diagnosis of "Acute myeloid leukemia, NOS" or "B lymphoblastic leukemia/lymphoma, NOS" may need to be given before the cytogenetic results are available or for cases that do not meet criteria for other leukemia subtypes.*

___ Histologic type cannot be assessed

Myeloproliferative Neoplasms
___ Chronic myelogenous leukemia, *BCR-ABL1* positive
___ Chronic neutrophilia leukemia
___ Polycythemia vera
___ Primary myelofibrosis
___ Essential thrombocythemia
___ Chronic eosinophilic leukemia, not otherwise specified (NOS)

+ Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.

___ Myeloproliferative neoplasm, unclassifiable

Mastocytosis
___ Systemic mastocytosis
___ Mast cell sarcoma

Myeloid/Lymphoid Neoplasms with Eosinophilia and Gene Rearrangement
___ Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement
___ Myeloid/lymphoid neoplasm with *PDGFRB* rearrangement
___ Myeloid /lymphoid neoplasm with *FGFR1* rearrangement

Myelodysplastic/Myeloproliferative Neoplasms
___ Chronic myelomonocytic leukemia
___ Atypical chronic myeloid leukemia, *BCR-ABL1* negative
___ Juvenile myelomonocytic leukemia
___ Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis
___ Myelodysplastic/myeloproliferative neoplasm, unclassifiable

Myelodysplastic Syndromes
___ Myelodysplastic syndrome with single lineage dysplasia
___ Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia
___ Myelodysplastic syndrome with ring sideroblasts and multilineage lineage dysplasia
___ Myelodysplastic syndrome with multilineage dysplasia
___ Myelodysplastic syndrome with excess blasts
___ Myelodysplastic syndrome with isolated del(5q)
___ Myelodysplastic syndrome, unclassifiable

Myeloid Neoplasms with Germline Predisposition
___ Acute myeloid leukemia with germline *CEBPA* mutation
___ Myeloid neoplasms with germline *DDX41* mutation
___ Myeloid neoplasms with germline *RUNX1* mutation
___ Myeloid neoplasms with germline *ANKRD26* mutation
___ Myeloid neoplasms with germline *ETV6* mutation
___ Myeloid neoplasms with germline *GATA2* mutation

Acute Myeloid Leukemia (AML) With Recurrent Genetic Abnormalities
___ Acute myeloid leukemia with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
___ Acute myeloid leukemia with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
___ Acute promyelocytic leukemia with *PML-RARA*
___ Acute myeloid leukemia with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
___ Acute myeloid leukemia with t(6;9)(p23;q34.1); *DEK-NUP214*
___ Acute myeloid leukemia with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
___ Acute myeloid leukemia (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*
___ Acute myeloid leukemia with mutated *NPM1*
___ Acute myeloid leukemia with mutated *CEBPA*

___ Acute myeloid leukemia with myelodysplasia-related changes

___ Therapy-related myeloid neoplasm

Acute Myeloid Leukemia, NOS#
___ Acute myeloid leukemia with minimal differentiation
___ Acute myeloid leukemia without maturation
___ Acute myeloid leukemia with maturation
___ Acute myelomonocytic leukemia
___ Acute monoblastic and monocytic leukemia

___ Pure erythroid leukemia
___ Acute megakaryocytic leukemia
___ Acute basophilic leukemia
___ Acute panmyelosis with myelofibrosis

Myeloid Proliferations Related to Down Syndrome
___ Transient abnormal myelopoiesis associated with Down syndrome
___ Myeloid leukemia associated with Down syndrome

___ Blastic plasmacytoid dendritic cell neoplasm

Acute Leukemias of Ambiguous Lineage
___ Acute undifferentiated leukemia
___ Mixed phenotype acute leukemia with t(9;22)(q34.1;q11.2); *BCR-ABL1*
___ Mixed phenotype acute leukemia with t(v;11q23.3); *KMT2A* rearranged
___ Mixed phenotype acute leukemia, B/myeloid, NOS
___ Mixed phenotype acute leukemia, T/myeloid, NOS
___ Mixed phenotype acute leukemia, NOS, rare types (specify type): _____________
___ Acute leukemia of ambiguous lineage, NOS

Precursor Lymphoid Neoplasms
___ B lymphoblastic leukemia/lymphoma, NOS[#]
___ B lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); *BCR-ABL1*
___ B lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A* rearranged
___ B lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
___ B lymphoblastic leukemia/lymphoma with hyperdiploidy
___ B lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL)
___ B lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH/IL3*
___ B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
___ B lymphoblastic leukemia/lymphoma, *BCR-ABL1*-like
___ B lymphoblastic leukemia/lymphoma with *iAMP21*
___ T lymphoblastic leukemia/lymphoma
___ Early T-cell precursor lymphoblastic leukemia

Mature B-Cell Neoplasms
___ Chronic lymphocytic leukemia/small lymphocytic lymphoma
___ B-cell prolymphocytic leukemia
___ Splenic marginal zone lymphoma
___ Hairy cell leukemia
___ Lymphoplasmacytic lymphoma
___ Intravascular large B-cell lymphoma
___ HHV8- positive DLBCL, NOS
___ Burkitt lymphoma

Mature T- and NK-cell Neoplasms
___ T-cell prolymphocytic leukemia
___ T-cell large granular lymphocytic leukemia
___ Aggressive NK-cell leukemia
___ Systemic EBV-positive T-cell lymphoma of childhood
___ Adult T-cell leukemia/lymphoma
___ Hepatosplenic T-cell lymphoma

Histiocytic and Dendritic Cell Neoplasms
___ Histiocytic sarcoma
___ Langerhans cell histiocytosis

+ Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.

___ Langerhans cell sarcoma
___ Interdigitating dendritic cell tumor
___ Follicular dendritic cell sarcoma
___ Fibroblastic reticular cell tumor
___ Disseminated juvenile xanthogranuloma

___ Other (specify): ____________________________

**+ Additional Pathologic Findings**
+ Specify: _______________________________________

**+ Cytochemical/Special Stains (Note D)**
+ ___ Performed
    + Specify stains and results: __________________________________
+ ___ Not performed

**Immunophenotyping (flow cytometry and/or immunohistochemistry) (Note E)**
___ Performed, see separate report: ___________________
___ Performed
    Specify method(s) and results: ______________________________
___ Not performed

**Cytogenetic Studies (Note F)**
___ Performed, see separate report: ___________________
___ Performed
    Specify method(s) and results: ______________________________
___ Not performed

**+ Fluorescence In Situ Hybridization (Note F)**
+ ___ Performed, see separate report: ___________________
+ ___ Performed
    + Specify method(s) and results: ______________________________
+ ___ Not performed

**+ Molecular Genetic Studies (Note F)**
+ ___ Performed, see separate report: ___________________
+ ___ Performed
    Specify method(s) and results: ______________________________
+ ___ Not performed

**+ Comment(s)**

## Explanatory Notes

### A. Specimen

Complete evaluation of hematopoietic disorders involving the bone marrow requires integration of data from multiple sources, including the clinical history, pertinent laboratory studies (eg, complete blood count [CBC], serum lactate dehydrogenase [LDH], and beta-2-microglobulin levels, serum protein electrophoresis, and immunofixation results), and a satisfactory peripheral blood smear and bone marrow specimen. In most instances, the latter entails a bone marrow aspirate specimen, an aspirate clot preparation (cell block), and a bone marrow core (trephine) biopsy. Touch preparations (imprints) of the biopsy specimen are also very helpful and highly recommended. The World Health Organization (WHO) classification recommends performing a 200-cell differential count on peripheral blood smears and a 500-cell differential count on bone marrow aspirate specimens in the evaluation of hematopoietic disorders. This will allow adequate evaluation of the cellular elements within the peripheral blood and bone marrow.

In addition, submission of appropriate bone marrow material for flow cytometry immunophenotyping, cytogenetic studies, fluorescence in situ hybridization (FISH), and molecular studies is often necessary. The guidelines that follow are suggested for handling of bone marrow specimens:

- The number of stained and unstained peripheral blood, bone marrow aspirate, and bone marrow core biopsy touch preparation smears should be recorded.
- The length of the bone marrow core biopsy(s) should be recorded.
- For conventional cytogenetic studies, a sterile bone marrow aspirate specimen received in a sodium heparin tube is ideal, but fresh specimens submitted in saline or RPMI transport medium may be appropriate.
- For immunophenotyping by flow cytometry, a bone marrow aspirate specimen received in an EDTA tube (lavender top tube) or an ACD tube (yellow top tube) is required.
- Decalcification: Bone marrow core biopsy specimens require decalcification, and care must be taken not to under- or over-decalcify the specimen, as that might impact the quality of histologic sections and could interfere with the outcome of immunohistochemical staining.
  - Acid decalcification procedures are most commonly done using formic acid.
  - EDTA decalcification circumvents DNA degradataion but is slower than acid-based decalcification, thus its use is thus more limited.
- Fixation:
  - Formaldehyde (formalin; 10% neutral buffered) is the most commonly used fixative for bone marrow samples; it is suitable for most ancillary tests, including molecular/genetic studies, colorimetric in-situ hybridization, and immunohistochemistry-based immunophenotyping.
  - Zinc formalin or B5 fixatives may produce superior cytologic detail but are not suitable for DNA extraction and may impair some immunostains. B5 has the additional limitation of requiring proper hazardous-materials disposal.
  - Over-fixation (ie, more than 24 hours in formalin, more than 4 hours in zinc formalin or B5) should be avoided for optimal immunophenotypic reactivity.

Care must be taken to ensure that high-quality specimens and sections are obtained for each bone marrow specimen. This often requires coordination with oncologists and radiologists in setting where they perform bone marrow sampling to ensure that appropriate triage of aspirate material is exercised.

### B. Aspiration/Biopsy Site

Bone marrow sampling (aspiration and core biopsy) is usually performed at the right and/or left posterior iliac crests. Aspirations and biopsies may be unilateral or bilateral, depending on the clinical indication for bone marrow sampling, as well as the preference of the oncology provider. Occasionally, the anterior iliac crest may be an appropriate choice in certain patients. The use of sternal aspirations is rare and should only be considered as a last resort option in very limited situations.

### C. Histologic Type

This protocol recommends assigning histologic type based on the 2017 WHO classification of hematolymphoid neoplasms.[1] Originally published in 2001 and revised and updated in 2008, this classification incorporates the morphologic, immunophenotypic, cytogenetic, and molecular findings into the final diagnosis. Whereas histologic

examination remains of paramount importance, many neoplasms will require the use of ancillary studies for precise diagnosis and subclassification. It may not be possible to provide a specific lymphoma diagnosis with bone marrow examination, particularly for patients in whom the bone marrow is the first identified site of involvement. While the list of the entities provided in the case summary covers the majority of primary bone marrow neoplasms, it is not exhaustive and omits rare and provisional entities in the 2017 WHO classification.

### D. Cytochemical/Special Stains

A number of cytochemical stains may be utilized in the evaluation of hematopoietic neoplasms involving the bone marrow. An iron (Prussian blue) stain is paramount in the evaluation of myelodysplastic syndromes and some myeloproliferative disorders. A reticulin stain is necessary for the assessment of myelofibrosis, a primary or secondary feature of many bone marrow neoplasms, most importantly myeloproliferative disorders. Cytochemical staining for myeloperoxidase is rapid, convenient, and helpful for the assessment of myeloid neoplasms. Other cytochemical stains for leukocyte alkaline phosphatase, and naphthol-ASD chloroacetate esterase may also be useful in certain conditions. Notwithstanding, cytochemical stains are no longer required for the diagnosis of most disorders.

### E. Immunophenotyping by Flow Cytometry and/or Immunohistochemistry

Immunophenotyping of bone marrow specimens can be performed by flow cytometry or immunohistochemistry. Each has advantages and disadvantages. Flow cytometry is rapid (hours), quantitative, and allows multiple antigens to be evaluated on the same cell simultaneously. Flow cytometry is a major tool for minimal residual disease detection in patients with acute leukemia. Antigen reactivity, however, cannot be correlated with architecture or cytologic features. In patients from whom a dry tap is obtained, an additional bone marrow core biopsy submitted fresh in transport medium may be disaggregated and utilized for flow cytometry immunophenotyping. Immunohistochemistry allows correlation of antigen expression with architecture and cytology. Not all antibodies are available for immunohistochemistry, particularly in fixed tissues, but one of its advantages is that it can be performed on archival tissue. Both techniques can provide diagnostic, prognostic, and therapy-guiding information.

### F. Cytogenetic and Molecular Genetic Studies

Cytogenetic and molecular data are integral to the evaluation of patients with primary bone marrow neoplasms. Cytogenetic analysis typically entails conventional karyotyping and FISH. Conventional karyotyping required viable cells. FISH may be performed on metaphase spreads from karyotyping studies or on air-dried, fresh unfixed aspirate or touch preparation slides. The use of array comparative genomic hybridization (aCGH) is used as an adjunct tool to detect copy number changes in certain conditions. Unlike conventional karyotyping, aCGH does not require viable cells. The use of molecular diagnostics is another central tool in the evaluation of many hematolymphoid malignancies. In addition to PCR-based techniques, molecular profiling using next-generation sequencing-based panels is increasingly available and provides valuable diagnostic, prognostic, and therapy-guiding data.

### References

1. Swerdlow S, Campo E, Harris N, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Geneva, Switzerland: WHO Press; 2017.