

## **Protocol for the Examination of Specimens from Patients with Hematopoietic Neoplasms Involving the Bone Marrow**

**Protocol applies to specimens from patients with hematopoietic neoplasms involving the bone marrow. This protocol does not apply to primary malignant bone tumors or bone marrow involvement by tumor arising from other primary sites.**

### **Based on:**

- CAP Cancer Protocol version 3.1.0.0
- CAP Protocol Web Posting Date: January 2018
- AAPA Macroscopic Examination Template Version 2.0
- AAPA Web Posting Date: October 2018

### **Revision History:**

None

### **Summary of Changes:**

None

### **Procedures Covered in this Protocol:**

- Bone Marrow Aspiration
- Bone Marrow Core (Trephine) Biopsy
- Peripheral Blood Smear
- Bone Marrow Aspirate Clot (cell block)
- Bone Marrow Touch Preparations (imprint)

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**Previous Lead Contributors:**

None

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The purpose of the Protocols is to support Laboratory Personnel engaged in the macroscopic examination of cancer resection specimens. The Protocols are based on specified relevant source documents, drafted by pathologists' assistant experts, and supported by information provided by the College of American Pathologists (CAP) and the American Joint Committee on Cancer (AJCC). These Protocols are intended to serve patients by ensuring that the macroscopic examination of cancer resection specimens is compliant with CAP Cancer Protocols, the AJCC Cancer Staging Manual, and provide optimization of the pre-analytic steps necessary to promote appropriate molecular studies.

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Utilization of the CAP Cancer Protocols at the Surgical Gross Bench**

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### **Molecular Considerations and Ancillary Testing:**

Submission of bone marrow (usually aspirate) material for flow cytometry immunophenotyping, cytogenetic studies, fluorescence in situ hybridization (FISH), and molecular studies is often necessary. Many acute leukemias are currently defined based upon their specific cytogenetic abnormalities. Care must be taken to evaluate the need for karyotype, FISH, or pertinent molecular studies at the time of sample procurement. FISH studies can be also performed on air-dried unfixed slides. In some cases, DNA can be scraped off air-dried unfixed slides for molecular studies. Since conventional karyotyping requires growing viable cells in culture, it is necessary to submit fresh specimens promptly to ensure the best opportunity for a successful study.

### **Cytogenetics:**

- For conventional cytogenetic studies, a bone marrow aspirate specimen received in a sodium heparin tube (green top tube) is ideal, but fresh specimens submitted in saline or RPMI transport medium are sufficient.

### **Flow Cytometry:**

- For immunophenotyping by flow cytometry, a bone marrow aspirate specimen received in an ACD tube (yellow top tube) or EDTA tube (lavender top tube) is preferred. Flow cytometry is a rapid, quantitative technique that allows for multiple antigens to be evaluated on the same cell simultaneously. Flow cytometry is also a primary technique for the detection of minimal residual disease in patients with acute leukemia.

### **Immunohistochemistry:**

- Immunohistochemistry is an immunophenotyping technique that requires formalin-fixed paraffin-embedded tissue sections and is performed most commonly on fully automated platforms; immunohistochemistry allows correlation of antigen expression with tissue architecture and cytomorphology.
- Documentation of expression of antigens such as CD20, CD33, or CD52 by the neoplastic cell population can play a role in the selection of potential targeted therapeutic options.
- A Prussian blue iron stain is an important component in the evaluation of bone marrow samples, both for semi-quantitative assessment of stainable iron stores and for the detection of ring sideroblasts.
- Reticulin and Trichrome stains are useful for the assessment of stromal fibrosis in bone marrow trephine biopsies, particularly in patients with myeloproliferative neoplasms.
- A myeloperoxidase stain is a rapid and convenient cytochemical stain that is useful for the detection of myeloblasts.
- A leukocyte alkaline phosphatase and naphthol-ASD chloroacetate esterase are less commonly utilized stains currently; they provide information related to cell origin or disease states.



**AAPA Macroscopic Examination Guidelines:  
Utilization of the CAP Cancer Protocols at the Surgical Gross Bench**

*These tests can be performed on formalin fixed paraffin embedded tissue sections. The macroscopic description should provide the fixative used. 10% neutral buffered formalin is the preferred fixative. It is recommended that the duration of the fixation be provided as well.*

## **PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:**

### **■ Procedures Covered by this Protocol:**

- Bone Marrow Aspirate
- Bone Marrow Core (Trephine) Biopsy
- Peripheral Blood Smear
- Bone Marrow Aspiration Clot (cell block)
- Bone Marrow Core Touch Preparation (imprint)

### **■ Specimen Site:**

- Bone marrow sampling is usually performed at the posterior iliac crest, either unilaterally or bilaterally.
- Occasionally, bone marrow sampling at the anterior iliac crest may be performed depending on the age of the patient.
- Sternal and tibial aspirations are discouraged, albeit they are still rarely performed.

### **■ Specimen Description:**

- Record the number of cores.
- Record the length and diameter of each core.
- Record the size of bone marrow clot.
- Record the number of stained and unstained peripheral blood, bone marrow aspirate, and bone marrow core biopsy touch preparation smears.

### **■ Specimen Adequacy, Protection, Fixation, and Decalcification:**

#### **• Adequacy:**

- The individual components extracted from a bone marrow biopsy procedure (bone marrow core biopsy sample, clot/aspirate, smears and imprints) may be separated for processing prior to receipt at the surgical gross bench. Nevertheless, while portions of the specimen may not be received at the surgical gross bench, efforts should be made to ensure that all necessary parts of the sample are received and routed to the appropriate labs / departments for processing. This should be managed through laboratory policies and procedures in coordination with the pathologist.

#### **• Protecting the specimen:**

- If received together, it is important to maintain separation between the smears, touch imprints, and other components; particularly those in fixatives, as fixative exposure is often deleterious to the smears / imprints.

#### **• Fixation and decalcification techniques:**

- 10% neutral buffered formalin is the preferred fixative for bone marrow samples. For bone marrow trephine biopsies, 10% formic acid is the preferred decalcification agent. This fixation approach retains suitability for most ancillary

tests such as molecular / genetic studies, in-situ hybridization, and immunophenotyping. Decalcification with EDTA produces minimal nucleic acid fragmentation and epitope alterations, but it is significantly slower than formic acid.

- Over-fixation (i.e., more than 24 hours in formalin, more than 4 hours in zinc formalin or B5) should be avoided for optimal immunophenotypic reactivity.
- Zinc formalin or B5 fixatives produce good 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.
- The use of Bouin's fixative is discouraged. It was previously recommended for lymphoid lesions because of improved preservation of nuclear detail (J Clin Pathol 2005;58:322–324. doi:10.1136/jcp.2004.019299 and Am J Dermatopathol. 1995 Oct;17(5):476-83).

**REFERENCE REVIEW:**

1. Khouri JD, Hussong JW, Arber MD, et al. Protocol for the Examination of Specimens from Patients with Hematopoietic Neoplasms Involving the Bone Marrow. *CAP Cancer Protocol, Bone Marrow* 3.1.0.0, 2018.
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