Protocol for the Examination of Specimens from Patients with Plasma Cell Neoplasms

Protocol applies to plasma cell neoplasms in bone marrow and extramedullary sites.

Based on:

AJCC/UICC TNM, 8th edition
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Revision History:

None

Summary of Changes:

This protocol is revised to match the 8th edition of the AJCC Cancer Staging Manual.

Procedures Covered in this Protocol:

- Bone Marrow (Trephine) Biopsy with Aspiration Clot and Peripheral Blood Smears
- Bone Core Biopsy
- Extraosseous/Nonosseous Sites:
  - Targeted Core Biopsies
  - Fine-Needle Aspirations
  - Excisional/Incisional Biopsy
  - Resection

Authors:

Renee Prew, PA(ASCP), HT(ASCP)CM*
Department of Pathology and Laboratory Medicine, VA Medical Center, Ann Arbor MI
Jennifer Davidson, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Courtney Hyland, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Darryl Kinnear, PA(ASCP)CM
Department of Pathology, Baylor College of Medicine, Houston, TX
John Lehman, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Stephanie Miller, PA(ASCP)CM
Providence Health & Services, Portland, OR
Tina Rader, PA(ASCP)CM
Drexel University College of Medicine, Philadelphia, PA
Erica Reed, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Mike Sovocool, MHS, PA(ASCP)CM
Pathology Associates of Syracuse, Syracuse, NY
Dennis Strenk, PA(ASCP)CM
Wisconsin Diagnostic Laboratories, Milwaukee, WI
Connie Thorpe, PA(ASCP)CM
Department of Pathology, Saint Louis University, St. Louis, MO
Jon Wagner, PA(ASCP)CM
Department of Pathology, Sutter Roseville Medical Center, Roseville, CA
Luke Wilson, PA(ASCP)CM

Mayo Clinic, Rochester, MN

*Denotes primary author. All other contributing authors are listed alphabetically.

Previous Lead Contributors:

None

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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Molecular Testing Techniques:

Cytogenetics:

RPMI complete culture media supplemented with a mitogen, phytohaemagglutinin, and antibiotics allowing lymphocytes to grow in culture. Submit a fresh portion of tissue sterilely in medium.
Green top (sodium heparin) vacutainer blood collection tube, 5-10 mL blood.

Snap-Frozen Tissue:

Optimal for DNA and RNA extraction. Snap freeze small samples of tissue (if adequate tissue is available), in dry ice, cold isopentane, or liquid nitrogen. Store at -70 to -80 °C until needed.

FISH & ISH:

Formalin-fixed paraffin-embedded (FFPE) tissue block or unstained slides (3-4 µm thick section on positively charges slides; two to three slides per marker requested) with a stained H&E slide (4-5 µm thick section). OR
Submit a fresh portion of tissue collected in RPMI culture media.
Green top (sodium heparin) vacutainer blood collection tube, 5-10 mL blood.

PCR and Sequencing:

Formalin-fixed paraffin-embedded (FFPE) tissue block or unstained slides (3-4 µm thick section on positively charges slides; two to three slides per marker requested) with a stained H&E slide (4-5 µm thick section). OR
Submit a fresh portion of tissue collected in RPMI culture media.
Lavender/purple top vacutainer blood collection tube, 5-10 mL blood.

Flow Cytometry Analysis:

RPMI culture media. Submit a fresh portion of tissue in medium.
Green top (sodium heparin) or lavender/purple top vacutainer blood collection tube, 5-10 mL blood.

Touch Preparations/Imprints:

Touch imprints may be made from the freshly cut surface, and the imprints fixed in 95% alcohol or air-dried. Unstained air-dried imprints can be used for Wright-Giemsa staining, cytochemistry, or fluorescence in situ hybridization (FISH).

Immunophenotype Considerations (immunohistochemistry, colorimetric ISH, flow cytometry immunophenotyping):

Immunopheynotyping of plasma cell neoplasms can be performed using immunohistochemistry and / or flow cytometry immunophenotyping. Neoplastic plasma cells are positive for CD38 and CD138, dim for CD45, and negative for CD19; in addition, CD20, CD56, and CD117 expression may be present in some cases. Constitutive overexpression of cyclin D1 is associated with the presence of t(11;14) or other genomic mechanisms such as polysomy of chromosome 11.

- Formalin-fixed paraffin-embedded (FFPE) tissue block or unstained slides (3-4 µm thick section on positively charges slides; two to three slides per marker requested) with a stained H&E slide (4-5 µm thick section).

Conventional Cytogenetic Analysis

Cytogenetic abnormalities are an important prognostic feature but are present in only 30-35% of patients with plasma cell myeloma. The deletion of chromosome 13 by cytogenetics or the presence of t(4;14), t(4;16), or -17p13 by FISH are all predictors of poor outcome. Cytogenetic abnormalities are categorized as hyperdiploid and hypodiploid (nonhyperdiploid). Hypodiploidy is an adverse prognostic feature, whereas hyperdiploidy is associated with better outcomes.

Plasma Cell Fluorescence In Situ Hybridization Recommendations by the International Myeloma working Group

Minimum Panel	t(4;14) (p16;q32) t(14;16) (q32;q23) del(17p13)
Comprehensive Panel	t(11;14)(q13;q32 -13 or del(13q) Ploidy category Chromosome 1 abnormalities

Plasma Cell Myeloma Cytogenetic Risk Groups

Unfavorable	Aneuploid or hypodiploid karyotype -13 or del(13q) t(4;14)(p16;q32) t(14;16)(q32;q23) del(17p13)
Favorable	Hyperdiploid karyotype t(11;14)(q13;q32)

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol:**

  - Bone Marrow – Trephine (core) biopsy (Random, Nontargeted)
    - Aspiration clot for histology
    - Aspiration clot in sodium heparinized green top tube for specialized tests
    - Peripheral blood smears
  - Bone - Core Biopsy (Targeted)
  - Extraosseous/Nonosseous Sites (Targeted)
    - Core biopsy
    - Incisional / Excisional biopsy
    - Resection
  - Fine-Needle Aspiration

- **Specimen Size and Extent of Resection:**

  - Bone Marrow – Trephine (core) biopsy
    - Provide length and diameter.
    - Specify location.
      - Right / left iliac crest
      - Sternum
  - Bone Marrow Aspiration Clot
    - Provide three dimensions of clot.
  - Bone – Core biopsy
    - Provide length and diameter.
  - Extraosseous Incisional / Excisional Biopsies
    - Provide three dimensions.

- **Specimen Integrity and Adequacy:**

  - The diagnosis of plasma cell neoplasms can be made from a sample obtained from a lesion involving bone or an extraosseous site.

  - Bone marrow aspirates tend to underestimate the extent of the disease, especially in cases associated with marked fibrosis.

  - If the specimen is inadequate or suboptimal for a definitive diagnosis, this information must be relayed to the clinician with what constitutes the specimen as inadequate or suboptimal.

- **Specimen Handling and Triage Recommendations:**

  - Other than bone marrow biopsies, specimens to rule out plasma cell neoplasia should always be submitted to the pathology department in the fresh state.
    - Some institutions suggest a rapid processing procedure with a goal of 4-hour turnaround time.

  - If the specimen quantity is insufficient to triage into all testing modalities, consult with the pathologist to determine how best to sample the specimen based on specimen amount,

specimen type, and patient history. This includes which fixation method / preservation media to use (B-5 fixative, zinc formalin, 10% neutral buffered formalin, RPMI culture media, cytogenetic culture media, etc.), and which specialized tests to request (flow cytometric analysis, molecular testing, cytogenetic analysis, etc.) for optimal results.

- Record the specimen size, color, consistency, the presence or absence or visible nodularity, hemorrhage, or necrosis.
- If possible, estimate time from excision to fixation.
- Sterile handling procedures should be utilized to prevent contamination of culture media which could impact specialized testing results.
- Touch imprints may be made from the freshly cut surface, and the imprints fixed in 95% alcohol or air-dried. Unstained air-dried imprints can be used for Wright-Giemsa staining, cytochemistry, or FISH. *
- A portion of the sample should be submitted in appropriate medium for cytogenetic analysis. *
- A portion of the sample should be submerged in an appropriate transport medium (such as RPMI) for flow cytometric analysis.
    - Immunophenotyping of plasma cell neoplasms can be performed using flow cytometry.
- A portion of the sample should be submerged in formalin for fixation.
    - Over-fixation (i.e., more than 24 hours) in formalin should be avoided for optimal immunophenotypic reactivity.
    - If more than one tissue fixative is used, take steps to ensure that the macroscopic description indicates the fixative used for each cassette submitted.

**Cytogenetic abnormalities are common in plasma cell myeloma. Neoplastic plasma cells often grow poorly in cell culture; therefore, fluorescence in situ hybridization (FISH) is commonly used to enhance the sensitivity of detecting cytogenetic abnormalities in plasma cell myeloma and is an important adjunct in prognostic assessment.*

TUMOR

TNM classification is not used when staging plasma cell neoplasms, as the tumor site is often unclear and differentiation between T, N, and M descriptors is not often possible. Diagnosis of plasma cell neoplasms involves staging, histologic type classification, and clinical prognostic factors and indices assessment.

- **Tumor Size: ***

  - Bone marrow biopsies and aspirates, core biopsies, and fine-needle aspiration:
    - Record appropriate measurements; OR
    - Unable to determine dimensions macroscopically.

  - Extraosseous/Nonosseous resection:
    - Solitary nodule – record measurement in three dimensions.
    - Multiple nodules – state the number of nodules present and provide a range in size in three dimensions.

  - Bone lesion(s) – provide three dimensions.

  *Tumor size may be determined from radiographic studies.

- **Tumor Site(s):**

  - Specify site of lesion resected, if identified.
    - Specify color, consistency, and identify any areas of necrosis.
      - Involved bone lesions often show a fish-flesh mass with hemorrhage and / or necrosis or have a soft, gelatinous, red appearance.

  - The bone lesions can be seen radiographically as “punched out” osteolytic defects in the vertebral bodies, skull, ribs, humerus, and femur, usually 1 to 4 cm in diameter.

  - Plasma cell neoplasms, particularly in extraosseous/nonosseous sites, should be distinguished from low-grade B-cell neoplasms with prominent plasmacytic differentiation.

- **PATHOLOGIC STAGING**

  Staging involves a combination of clinical, radiologic and surgical data and is staged the same for both children and adults. The criteria for the diagnosis of plasma cell myeloma include the presence of the triad of monoclonal bone marrow plasma cells, M-protein in the serum and / or urine, and the presence of related organ or tissue impairment (CRAB: hypercalcemia, renal insufficiency, anemia, and / or bone lesions).

Durie-Salmon Staging System for Plasma Cell Myeloma:

Stage I	Low M-protein levels (IgG <50 g/L; IgA <30 g/L) Urine Bence-Jones protein <4 g/24 hours Absent or solitary bone lesions Normal hemoglobin, serum calcium, and non-M-protein Ig levels
Stage II	Overall values between stages I and III
Stage III	High M-Protein levels (IgG >70 g/L; IgA >50 g/L) Urine Bence-Jones protein >12 g/24 hours Multiple lytic bone lesions Hemoglobin <8.5 g/dL; serum calcium >12 mg/dL

Note:
Patients are further subclassified based on renal function; A=serum creatinine <2 mg/dL; B=serum creatinine >/=2 mg/dL.
Any one or more of the listed corresponding abnormalities fulfill the criteria for stage III.

Revised International Staging System for Plasma Cell Myeloma:

RISS stage group	Factors
Stage I	Serum β_2-microglobulin <3.5mg/L *and* serum albumin ≥3.5g/dL *and* no high-risk cytogenetics* *and* normal LDH
Stage II	Not stage I or III
Stage III	Serum β_2-microglobulin ≥5.5mg/L *and* high-risk cytogenetics* *and/or* high LDH

*High-risk cytogenetics consists of one or more of the following:
Del 7p, t(4;14), or t(14;16).
Note: The following variables must be collected at the time of diagnosis for staging of plasma cell myeloma according to the RISS:
Serum β_2-microglobulin, serum albumin, serum LDH, and FISH results from the bone marrow specimen for t(4;14), t(14;16), and del17p.

- **HISTOLOGIC TYPE** (2008 WHO classification)

  Universal, consensual world collaboration on the classification of plasma cell neoplasms, based on morphological diagnostic criteria, immunophenotypic, genetic features, clinical and prognostic relevance.

  World Health Organization Classification of Plasma Cell Neoplasms

  - Plasma cell myeloma
  - Solitary plasmacytoma of bone
  - Extraosseous plasmacytoma
  - Monoclonal immunoglobulin deposition diseases
    - Primary amyloidosis
    - Systemic light and heavy chain deposition diseases

- **CLINICAL and LABORATORY DATA:**

  Prognostic Factors:
  - Clinical History
  - Physical Examination
  - Biopsy (preferable excisional) with interpretation by qualified pathologists
  - Immunophenotype Testing
  - Cytochemistry
  - Flow Cytometry Immunophenotyping
  - Fine-Needle Aspiration
  - Hematologic Findings:
    - Bone marrow biopsy (core, aspiration), complete blood (CBC) and platelet count findings, erythrocyte sedimentation rate or CRP, and peripheral blood smear
  - Imaging Screening Procedures:
    - X-Ray – Skeletal survey is essential, especially in patients suspected of myeloma
    - Computed tomography (CT) – Usually not required due to standard skeletal surveys that usually detect majority of lesions
    - Magnetic Resonance Imaging (MRI) – Superior to CT in detecting soft tissue lesions; useful in assessing spinal cord compressions and extramedullary type lesions
  - Lytic bone lesion detected
  - Chemistry Panel Findings:
    - M-protein detected in serum or urine
    - Hypercalcemia
    - Serum creatinine elevation
    - Anemia
    - Elevated serum beta-2-microglobulin
      - <3.5 mg/L
      - ≥3.5 mg/L to <5.5 mg/L
      - ≥5.5 mg/L
    - Serum albumin ≥3.5 g/dL
  - Clinical Features:
    - Bone pain or pathologic fracture
    - Anemia
    - Recurrent infection
    - Hypercalcemia
    - Hyperviscosity

- Renal failure
- Spinal cord compression (10%)
- Peripheral neuropathies caused by amyloidosis

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