Protocol for the Examination of Specimens from Pediatric Patients with Ewing Sarcoma

Protocol applies to pediatric patients with osseous and extraosseous Ewing sarcoma family of tumors, including peripheral primitive neuroectodermal tumor. This protocol does not apply to adult Ewing sarcoma.

Based on:

AJCC/UICC TNM 8th edition
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Revision History:

None

Summary of Changes:

This protocol is revised to the 8th edition of the AJCC Cancer Staging Manual and the current version of the CAP Cancer Protocol EwingSarcoma 3.2.0.2.

Procedures Covered in this Protocol:

- Biopsy
- Resection

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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.

The AAPA cautions that the use of the Protocols in practice may require the use of additional considerations that are beyond the scope of the Protocols. The AAPA does not offer medical advice or diagnoses, or engage in the practice of medicine. The information provided in the Protocols is not intended or implied to be a substitute for the Laboratory Personnel's own training, professional medical opinion, diagnosis, or treatment advice. All content, including text, graphics, images and information contained in the Protocols are for the above stated purposes only. Laboratory Personnel are encouraged to confirm any information provided in these Protocols with other sources. The inclusion of a product name, organization, or service in an AAPA publication, including without limitation the Protocols, should not be construed as an endorsement of such product, organization, or service, nor is failure to include the name of a product, organization or service to be construed as disapproval.

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Immunohistochemistry Considerations:

First priority should always be given to formalin fixed tissue for histomorphologic evaluation. Nearly all Ewing sarcoma tumors are positive for CD99 in cases with a membranous pattern. CD99 should be done in a panel with muscle markers, neural markers, epithelial markers, and lymphoid markers. Vimentin is consistently positive in Ewing Sarcoma. FLI1 can be exhibited in other tumor types, but can be sensitive in Ewing sarcoma.

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 fixation be provided as well.

Classification of many subtypes of sarcoma is not dependent upon special studies, such as cytogenetics or molecular genetics, but frozen tissue may be required to enter patients into treatment protocols. Discretion should be used in triaging tissue from sarcomas. Adequate tissue should be submitted for conventional light microscopy before tissue has been snap frozen, submitted for cytogenetics, or electron microscopy.

Molecular Considerations:

- Snap freeze a minimum of 100 mg of viable tumor and store at -70 degrees C.

Snap frozen tissue may offer increased sensitivity of detection. Therefore, additional special studies (e.g., cytogenetics, fluorescence in situ hybridization [FISH], reverse transcriptase polymerase chain reaction) are critical to the molecular workup of Ewing sarcoma. These tests require at least 100 mg of viable, fresh, or snap frozen tissue stored at -70 degrees Celsius as the second priority for workup. In the case of limited tissue, the frozen tissue aliquot (stored at -70 °C) or touch preparations made from fresh tissue can be used for RT-PCR or FISH.

FISH Evaluation:

- Molecular testing on formalin fixed paraffin embedded tissue may be performed for FISH evaluation of *EWSR1* rearrangement and for RT-PCR evaluation of *EWSR1-FLI1, EWSR1-ERG*, and other Ewing sarcoma translocations.

Cytogenetic Considerations:

- Submit fresh tumor in alpha MEM transport media or other suitable media for cytogenetic analysis.

Electron Microscopy:

- Submit 1 mm cubes of fresh tumor in cold 2% glutaraldehyde for electron microscopy.

Electron microscopy may also be valuable in the evaluation of Ewing sarcoma.

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol: ***

  - Core needle biopsy
  - Incisional biopsy
  - Excisional biopsy
  - Resection
  - Other (specify)

**This protocol should not be used for adult Ewing sarcoma. Ewing sarcoma in adults may be treated differently than pediatric Ewing sarcoma, and use of the AJCC TNM staging system remains appropriate for these patients.*

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

  Biopsy (Needle, Incisional, Excisional):

  - Measure the specimen in three dimensions.
  - Core needle biopsies can obtain sufficient material for special studies and morphologic diagnosis.
  - Open incisional biopsy is generally the preferred and most widely used technique as it consistently provides a larger sample of tissue and maximizes the opportunity for a specific pathologic diagnosis.
  - Excisional biopsy may not include an adequate margin of normal tissue, even with an operative impression of total gross removal.

- **Explanatory Note:**

  In cases of nonexcisional biopsy (e.g., core biopsy, incisional biopsy), the tumor size cannot be determined on pathologic grounds; consequently, imaging data (computed tomography, magnetic resonance imaging, etc.) may be used instead.

  Resection:

  The extent of resection specimens may be intralesional, marginal, segmental/wide, or radical.

  - *For all types of resections, orientation of the specimen and application of ink prior to sectioning are highly recommended*.
  - Intralesional resections extend through tumor planes, with macroscopic or microscopic residual tumor at the surgical margins.
  - Marginal resections involve a margin formed by reactive tissue surrounding the tumor.
  - Segmental/Wide resections are intracompartmental resections. A single portion of bone is resected including the lesion and a cuff of normal bone.
  - Radical resections involve the removal of entire bone, or the excision of the adjacent muscle groups if the tumor is extracompartmental.
  - If specimen is an amputation or an en-bloc resection, measure and describe all structures attached.

  Full representative mapping of the specimen is recommended (See Tumor Mapping Section).

- **Specimen Integrity:**

  Statements should include, with as much clarity as can be provided, the anatomic location of the defects or disruptions, and should also incorporate statements which assess the relationship of any defects or disruptions to the tumor and final surgical margin. If defects or disruptions involve the tumor or margins, and serve to hinder assessment of the final surgical margin this must be stated. Consultation with the surgeon for clarification should be considered, as well as differentially inking the margin in areas affected by defects or disruptions. Completely fragmented tumors, which cannot be reasonably measured, should be accompanied with a maximal tumor dimension on the requisition sheet or through consultation with the surgeon.

TUMOR ("T" of TNM)

The Ewing sarcoma family of tumors includes both peripheral primitive neuroectodermal tumor (PNET) and Ewing sarcoma (ES) which occur both in children and adults. The malignancy may occur in both bone and soft tissue sites including unusual sites such as skin or leptomeninges. Because PNET/ES can occur in both bone and soft tissue, the AJCC/UICC TNM staging systems for bone and soft tissue based tumor may be used for pathologic staging if desired and can be reported in the comment section.

Definition of Primary Tumor (pT)	
For Primary Osseous Tumors (Appendicular Skeleton, Trunk, Skull, and Facial Bones)	
pT Category	**pT Criteria**
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pT1	Tumor ≤8 cm in greatest dimension*
pT2	Tumor >8 cm in greatest dimension
pT3	Discontinuous tumors in the primary bone site

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**The size of ≤8 cm in greatest dimension is a critical threshold. Ewing sarcoma patients with a tumor ≤8 cm in greatest dimension have a better prognosis than those with a tumor >8 cm.*

For Primary Extraosseous Tumors (Soft Tissue, Trunk and Extremities)	
pT Category	**pT Criteria**
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pT1	Tumor 5 cm or less in greatest dimension
pT2	Tumor more than 5 cm and less than or equal to 10 cm in greatest dimension
pT3	Tumor more than 10 cm and less than or equal to 15 cm in greatest dimension
pT4	Tumor more than 15 cm in greatest dimension

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TNM Descriptors:

For identification of special cases of TNM or pTNM classifications, the "m" suffix and the "y" and "r" prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis.

The "m" suffix indicates the presence of multiple primary tumors in a single site and is recorded in parentheses: pT(m)NM.

The "y" prefix indicates those cases in which classification is performed during or following initial multimodality therapy (i.e., neoadjuvant chemotherapy, radiation therapy, or both chemotherapy and radiation therapy). The cTNM or pTNM category is identified by a "y" prefix. The ycTNM or ypTNM categorizes the extent of tumor actually present at the time of that examination. The "y" categorization is not an estimate of tumor prior to multimodality therapy (i.e., before initiation of neoadjuvant therapy).

The "r" prefix indicates a recurrent tumor when staged after a documented disease-free interval, and is identified by the "r" prefix: rTNM.

Restaging of Recurrent Tumors:

The same staging should be used when a patient requires restaging of sarcoma recurrence. These reports should specify whether patients have primary lesions or lesions that were previously treated and have subsequently recurred. The identification and reporting of etiologic factors such as radiation exposure and inherited or genetic syndromes are encouraged. A tissue biopsy to confirm diagnosis prior to initiation of therapy should be included in the workup for recurrent sarcoma.

- **Tumor Size:**

  Biopsy:
  - Measure in three dimensions.

  Resection:
  - Measure in three dimensions or length and maximum circumference of limbs.
  - State if tumor is multifocal or discontinuous from the primary site (skip metastasis).

  Extraosseous Ewing Sarcoma / PNET is often large at presentation, ranging from 1 to 40 cm in diameter and frequently larger than 10 cm.

- **Tumor Site(s):**

  - Ewing sarcoma develops in the diaphysis or metaphysis of long bones, but can occur in any bone.
    - Most often femur, tibia, and humerus
    - Also seen in scapulae, ribs, and pelvic bones

  - Extraskeletal Ewing sarcoma
    - Commonly seen in the paravertebral region, chest wall, and soft tissue of the extremities.
    - Ewing sarcoma can also be seen in the soft tissues of the trunk, head, neck, and viscera

- **Tumor Anatomic Location and Extent of Local Involvement:**

  Extent of Osseous Tumors (describe all that apply)
  - Specify the tumor location in the bone:
    - Diaphysis
    - Metaphysis
    - Medullary cavity
    - Periosteal
  - Specify if the tumor erodes through cortex and extends into soft tissue (compression or true invasion).
  - Specify if there is extension through the epiphyseal plate; extension into or across the joint space.
  - Specify if vascular involvement is seen.
  - Specify if skip metastases are seen.
  - Other (specify).

Extent of Primary Extraosseous Tumors (describe all that apply)

- Dermal
- Subcutaneous / suprafascial
- Subfascial
- Intramuscular
- Intra-abdominal / pelvic
- Retroperitoneal
- Chest wall
- Other (specify)

- **Tumor Mapping:** *(Figure 1)*

  - Radiograph the intact specimen to determine in which plane to cut the bone slab.
    - This step is optional, as radiography is not accessible at some institutions.
  - Apply ink to the external surface, and submit soft tissue margins and bone marrow margins.
  - Freeze the specimen, or fix in 10% neutral buffered formalin.
  - Incise the soft tissue in a plane that will demonstrate the greatest extent of the tumor.
  - Bisect the specimen with a band saw.
  - Cut a 0.5 cm thick parallel section through the bone.
  - Photograph, radiograph, or photocopy (in a sealed plastic bag) the bone slab.
  - This central full face of the specimen and lesion is mapped by drawing a grid on the radiograph or photograph; the entire cross-section is mapped and submitted for microscopic examination and estimation of percentage of tumor necrosis.
  - *After formalin fixation and decalcification*,* sections are taken to demonstrate the tumor, relationship to adjacent normal bone, invasion of contiguous structures, and margins.
  - The location of sections taken is indicated on the diagram of the specimen.
  - The entire cross-section is sampled and correlated with the radiograph.

  *Formic acid-formaldehyde decalcification solution is recommended, as it provides a more gentle decalcification method and allows for retention of antigens for immunohistochemistry and preservation of DNA for possible molecular studies on formalin fixed paraffin embedded tissue.

- **Explanatory Notes:**

  Bone dust can create artifacts that may be difficult to interpret. Sections should be oriented so that the portion cut by the histotechnologist will be opposite the side cut by the saw such as applying ink to one side and indicating the appropriate side to be sectioned. Alternatively, bone dust can be cleaned off with a brush or by rinsing in saline.

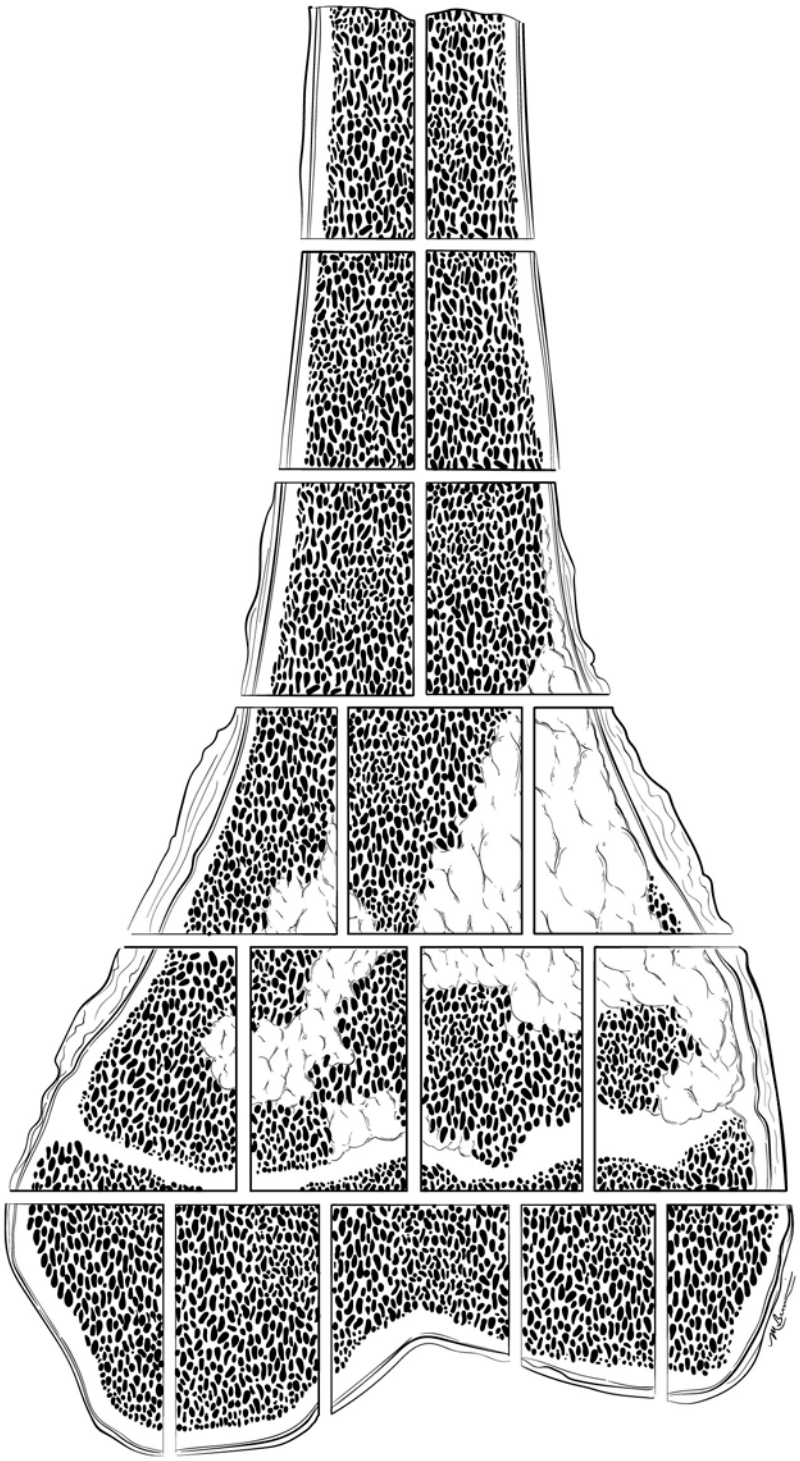

Figure 1: Bone tumor map/grid demonstrating histologic sections.

- **Tumor Assessment, Macroscopic Features:**

  - Describe the macroscopic features of the tumor including: *
    - Measurement: provide three dimensions.
    - Note the appearance including color, bone formation and/or cartilage formation.
    - Percentage of necrosis (areas that appear necrotic may be myxoid or edematous).
    - Bone destruction.
    - Intramedullary and soft tissue involvement (often greater than suggested on radiographs).
    - State the color and consistency - gray, tan, or white, poorly demarcated, fleshy, friable, or mucoid.
    - Identify if the tumor is circumscribed or infiltrating, multinodular, or lobulated.
    - Identify areas of hemorrhage, cystic degeneration, and necrosis.
    - Identify the presence or absence or a capsule or pseudocapsule.

  *The macroscopic features are altered in most cases due to the administration of chemotherapy prior to resection. It may be difficult to identify any gross tumor.

- **Margins:**

  Excisional Biopsy:

  - Provide the distance of tumor from closest bone margin in cm.
  - Provide the distance of tumor from closest soft tissue margin in cm.
  - Provide the distance of tumor from the closest other (e.g., parenchymal) margin in cm.
  - Specify the margin that is macroscopically involved by tumor.

  The extent of resection (i.e., gross residual disease versus complete resection) has the strongest influence on local control of malignancy.

  Resection:

  - Describe bone, soft tissue, marrow involvement by tumor.
  - Provide the distance of tumor from the closest bone margin in cm.
  - Provide the distance of tumor from the closest soft tissue margin in cm.
  - Provide the distance from the closest other (e.g., parenchymal) margin in cm.
  - Specify the margin that is macroscopically involved by tumor.
  - Submit soft tissue margins for limb resections.
  - State if the neurovascular bundle at the margin is involved or uninvolved.

- **Explanatory Notes:**

  The adequacy of margins of resection is an important determination in the resection specimen because it affects decisions regarding adjuvant therapies. Positive or proximate surgical margins are generally managed by radiotherapy.

  In the current Children's Oncology Group study of Ewing Sarcoma, the following margins are considered adequate:

  - Bone margin: 2 cm to 5 cm

- Fascia, periosteum, and intermuscular septa: 2 mm
- Fat, muscle, and medullary bone: 5 mm

The Children's Oncology Group states that any tumor at the margin, either viable, nonviable, or treated, is considered positive. There is no current consensus as to whether margins involved by treated tumor require further treatment.

In the setting of Ewing sarcoma involving an encapsulated organ, surgical margins are considered negative if the organ's capsule is not surgically breached by tumor.

Other Primitive Neuroectodermal Tumors:

Askin Tumors

Askin tumors involve the soft tissue of the chest wall and/or the ribs, without conclusive evidence of origin from bone. These tumors should be denoted as "chest wall."

Central Nervous System (CNS) PNET and Intracranial Ewing Sarcoma

Medulloblastomas and primitive neuroectodermal tumors of the CNS are pathologically different neoplasms and should not be included in this protocol. Intracranial Ewing sarcoma originating in non-CNS soft tissues such as the meninges should be included in this protocol.

LYMPH NODES ("N" of TNM)

- **Lymph Nodes:** (if applicable)

The AJCC/UICC TNM staging systems for bone and soft tissue based tumor may be used for pathologic staging if desired and can be reported in the comment section.

Definition of Regional Lymph Node (pN)

pN Category	pN Criteria
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Regional lymph node metastasis

Lymph Node Submission:

- Specify number of lymph nodes examined.
- Specify number of lymph nodes involved.

Regional and Nonregional Lymph Nodes:

- Count lymph nodes identified.
- Specify site of lymph nodes identified (regional, nonregional).
- Measure lymph nodes in three dimensions.
- Describe the cut surface of the identified lymph nodes.
- Submit all lymph nodes for microscopic examination.
    - Submit small lymph nodes in toto.
    - Serially section and entirely submit larger macroscopically negative lymph nodes.
    - Representative sections from macroscopically positive nodes are adequate.
- If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.
- Take steps to ensure that an accurate lymph node count can be rendered.

- **Explanatory Notes:**

- Lymph node involvement is rare in bone sarcomas.
- Staging of lymph nodes as NX is equivalent to N0 stage grouping.
    - Due to the rarity of lymph node involvement in bone sarcomas, the designation NX may not be appropriate and cases should be considered N0 unless clinical node involvement is clearly evident.
    - Patients whose nodal status is not determined to be positive for tumor, either clinically or pathologically, should be designated as N0.
- Presence of positive nodes (N1) in M0 tumors is considered stage III (extraosseous only).

METASTASIS ("M" of TNM)

- **Metastasis:**

The AJCC/UICC TNM staging systems for bone and soft tissue based tumor may be used for pathologic staging if desired and can be reported in the comment section.

Definition of Distant Metastasis (pM) (required only if confirmed pathologically)

For Primary Osseous Tumors	
pM Category	**pM Criteria**
M0	No distant metastasis
pM1	Distant metastasis
pM1a	Lung
pM1b	Bone or other distant site

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For Primary Extraosseous Tumors (Soft Tissue, Trunk and Extremities)	
pM Category	**pM Criteria**
M0	No distant metastasis
pM1	Distant metastasis

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The presence or absence of metastatic disease is the primary factor in the staging and treatment of pediatric patients with Ewing sarcoma.

- **Explanatory Notes:**

  - Any metastatic disease defined by standard imaging techniques or bone marrow aspirate / biopsy by morphology is an adverse prognostic factor. The presence or absence of metastatic disease is the single most powerful predictor of outcome. Metastases at the time of diagnosis are detected in about 25% of patients.

  - Patients with metastatic disease confined to the lung have a better prognosis than do patients with extrapulmonary metastatic sites. Patients with solitary lung metastasis have a better prognosis than those with multiple lung lesions. Consequently, it is important to document the number of lung metastases.

  - Bone metastases carry a worse prognosis than do lung metastases.

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