Protocol for the Examination of Specimens from Pediatric Patients with Rhabdomyosarcoma

Protocol applies to all rhabdomyosarcoma variants and Ectomesenchymoma. This protocol does not apply to adult rhabdomyosarcoma.

AJCC/UICC TMN staging systems do not apply to rhabdomyosarcoma. The Intergroup Rhabdomyosarcoma Study Postsurgical Clinical Grouping System is recommended.

Based on:

CAP Cancer Protocol version 3.2.0.2
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Revision History:

None

Summary of Changes:

This protocol is to the current version of the CAP Cancer Protocol Rhabdomyosarcoma 3.2.0.2.

Procedures Covered in this Protocol:

- Biopsy
- Resection

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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.

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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Molecular Considerations:

Special studies are critical to the molecular work-up of rhabdomyosarcoma.

- First priority should always be given to formalin-fixed tissue for morphologic evaluation.
- A minimum of 100 mg of viable, fresh tumor should be snap-frozen and kept at -80 degrees Celsius for potential molecular studies.
- If tissue is limited, the frozen tissue aliquot used for frozen section may be kept at -80 degrees Celsius for potential molecular studies.
- Obtain touch preparations from frozen section tissue. *
- If no other tissue is available, FISH may be performed on paraffin sections.

*Translocations, which are typical of the alveolar type of rhabdomyosarcoma may also be detected using molecular studies to evaluate fusion status, reverse transcriptase polymerase chain reaction (RT-PCR), or fluorescence in situ hybridization (FISH) and may be performed on paraffin sections or frozen tissue. When minimal material is submitted, FISH can also be performed on touch preparations made from fresh material obtained at the time of biopsy.

Alveolar rhabdomyosarcoma is a poor prognosis subtype with a 53% 5-year survival.

RT-PCR for PAX3- and PAX7-FOXO1 fusion gene products occur in approximately 85% of alveolar rhabdomyosarcoma cases, and testing for these gene products is recommended for difficult cases.

Cytogenetics:

The incidence of t(1;13) (resulting in a PAX7-FOXO1 gene fusion) and t(2;13) (PAX3-FOXO1 gene fusion) is strongly correlated with alveolar rhabdomyosarcoma. The embryonal and pleomorphic subtypes of rhabdomyosarcoma are genetically heterogeneous and lack evidence of these gene fusions (with rare exceptions).

Immunohistochemistry:

Immunohistochemical stains including myogenin, desmin, Myo-D1, cytokeratin, CD99, WT1, synaptophysin, chromogranin, and leukocyte common antigen will distinguish alveolar rhabdomyosarcoma from malignant small round cell tumors.
Alveolar rhabdomyosarcoma shows diffuse and strong nuclear staining for myogenin.

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.

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol:**

  - Biopsy:
    - Core needle
    - Incisional
    - Excisional

  - Resection:
    - Marginal resection
    - Wide local resection
    - Radical resection
    - Amputation
    - Other (specify)

- **Specimen Laterality:**

  - Right
  - Left
  - Midline

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

  - Measure the specimen in three dimensions.
  - If the specimen is an amputation or an en-bloc resection, measure and describe all structures attached.

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

  - For all types of resections, orientation of the specimen is critical, and application of ink is strongly recommended.
  - Intralesional resections extend through tumor planes, with macroscopic or microscopic residual tumor at the surgical margins.
  - Marginal resections involve a margin formed by inflammatory tissue surrounding the tumor.
  - Wide, radical resections have surgical margins that extend through normal tissue, usually external to the anatomic compartment containing the tumor.

- **Specimen Integrity, and Adequacy:**

  The extent of resection (i.e., gross residual disease versus complete resection) has the strongest influence on local control of malignancy. Consequently, the adequacy of resection and integrity of the surgical margin are crucial. If defects exist that hinder definitive assessment of the margin, care must be taken to reconcile the defects and reacquire true – false margin relationships. Correlation with the surgeon is suggested and when uncertainty persists, differentially inking the margins and stating the exact location of the defect may provide a significant contribution.

Modified Site, Size, Metastasis Staging for Rhabdomyosarcoma (for relevant stage) *

Note: Clinical information required to definitively assign stage (e.g., nodal status or distant metastatic disease) may not be available to the pathologist.

Stage I	(requires all the following to be true) • Tumor involves favorable site (i.e., bile ducts, orbit, head and neck, or genitourinary site [excluding bladder, prostate and cranial parameningeal]). • Tumor metastatic to distant site not identified.
Stage II	(requires all the following to be true) • Tumor involves unfavorable site (i.e., bladder/prostate, extremity, parameningeal or other site not mentioned in stage I). • Tumor size ≤5 cm. • Tumor involvement of lymph nodes not identified. • Tumor metastatic to distant site not identified.
Stage III	(requires that one of the following be true) • Tumor involves unfavorable site, is ≤ 5 cm, and involves regional lymph nodes, but distant metastases are not identified. • Tumor involves unfavorable site and is >5 cm, with or without regional lymph node involvement, but distant metastases are not identified.
Stage IV	• Distant metastases are required to be present.

* Traditional TMN staging is not used for rhabdomyosarcomas, rather modified grouping and staging systems are to be followed.

The Intergroup Rhabdomyosarcoma Study Postsurgical Clinical Grouping System

Note: Clinical information required to definitively assign stage group (e.g., gross residual disease or distant metastatic disease) may not be available to the pathologist at the time of macroscopic examination. Alternatively, this protocol may not be applicable to some situations (e.g., group IIIA). If applicable, the appropriate stage group may be assigned by the pathologist.

Group I
- A Localized tumor, confined to site of origin, completely resected
- B Localized tumor, infiltrating beyond site of origin, completely resected

Group II
- A Localized tumor, gross total resection, but with microscopic residual disease
- B Locally extensive tumor (spread to regional lymph nodes), completely resected
- C Locally extensive tumor (spread to regional lymph nodes), gross total resection, but microscopic residual disease

Group III
- A Localized or locally extensive tumor, gross residual disease after biopsy only
- B Localized or locally extensive tumor, gross residual disease after major resection (greater than 50% debulking)

Group IV

Any size primary tumor, with or without regional lymph node involvement, with distant metastases, without respect to surgical approach to primary tumor

TUMOR

- **Tumor General Descriptors and Considerations Specific to Rhabdomyosarcoma:**

  - Identify the presence or absence of softening consistent with necrosis.
    - If softening/necrosis is present, approximate the percentage of tumor involved, based on serial slices.

    The significance of necrosis in pretreated soft tissue sarcomas is unknown, but the extent of necrosis in such specimens is not used as a grading parameter.

  - Describe the appearance and texture of tumor cut surface, including, but not limited to:
    - Color
    - Firm or soft
    - Gelatinous
    - Calcified
    - Hemorrhagic
    - Botryoid
    - Nodular

  - Identify any involvement or invasion of major structures (e.g., nerve, bone, major blood vessels).

  - Assess for the presence of satellite nodules away from primary tumor.
    - Measure distances to the nearest resection margin.

  - Sample at minimum one section for each centimeter of the tumor.

  - Tissue taken for special studies (e.g., electron microscopy, snap-freezing, cytogenetic analysis, DNA flow cytometry) should be documented in the macroscopic description.

- **Tumor Size:**

  - Include three dimensions.
  - If there are multiple tumors, include three dimensions for each.

- **Tumor Site(s): ***

  - Bile duct
  - Bladder/prostate
  - Cranial/parameningeal
  - Extremity
  - Genitourinary (not bladder/prostate)
  - Head and neck (excluding parameningeal)
  - Orbit
  - Other(s) (includes trunk, retroperitoneum, etc) (specify)

  *Botryoid rhabdomyosarcoma, a prognostically favorable subtype and a variant of embryonal rhabdomyosarcoma, develops in the walls of hollow, mucosal-lined structures, such as the bladder, vagina, nasopharynx, and common bile duct.

Macroscopically, most embryonal rhabdomyosarcomas of the urinary bladder present as polypoid, soft, myxoid intraluminal masses resembling a cluster of grapes (botryoid).

- **Tumor Depth and Extent for Soft Tissue-Based Tumors:**

  - Dermal
  - Subcutaneous
  - Subfascial
  - Intramuscular
  - Intra-abdominal
  - Retroperitoneal
  - Intracranial
  - Organ based
  - Other (specify)

- **Margins:**

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

  - Specify if margins are uninvolved or involved by tumor.
  - Measure the distance of tumor from the closest margin in cm; specify the margin.
  - All margins less than 2 cm should be measured and specified in the final report.
  - Most surgical excision of extremity rhabdomyosarcoma specimens or limb salvage specimens will have 6 margins:
    - Superficial and deep
    - Proximal and distal
    - Medial and lateral
  - Specify whether a margin consists of either a fascial layer, periosteum or other anatomic barrier (e.g., diaphragm).

  - **Explanatory Notes:**

    - The definition of what constitutes a sufficiently “wide” margin of normal tissue in the management of rhabdomyosarcoma has evolved over time from resection of the entire muscle to a resection with a 2 cm to 3 cm margin. However, this is not a universal definition.

LYMPH NODES

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

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

  Depending on the location of the specimen, lymph nodes may or may not accompany the specimen.

  If lymph nodes are encountered:
  - Count lymph nodes identified.
  - Specify site of lymph nodes identified.
  - Measure lymph nodes in three dimensions.
    - For multiple lymph nodes, provide a size range in greatest dimension.
  - 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 lymph nodes.
  - 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.

METASTASIS

- **Metastasis:**
  - Specify site of distant metastasis if known.
  - The most common sites of metastases include lung, bone, bone marrow, and distant lymph nodes.
  - Uncommon sites of metastases include brain, liver, and spleen.

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