# Protocol for the Examination of Specimens from Pediatric Patients with Hepatoblastoma

## Protocol applies to hepatoblastoma. Other malignant primary hepatic tumors are not included.

**No AJCC/UICC TNM Staging System**
**The Children's Oncology Group Staging System is recommended**

**Based on:**

CAP Cancer Protocol version: Hepatoblastoma 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 Hepatoblastoma 3.2.0.2.

**Procedures Covered in this Protocol:**

- Biopsy
- Hepatectomy, Partial or Complete

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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.

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**Molecular Considerations:**

- **Freeze 100 mg (minimum) or up to 1 g of tumor and normal liver.**

First priority should be given to formalin fixed tissue for morphologic evaluation and immunohistochemistry. Numerous recent studies have documented molecular genetic abnormalities in hepatoblastomas. Collection of fresh or frozen hepatoblastoma tumor from macroscopically different regions as well as non-tumoral liver tissue (up to 1 g with a minimum of 100 mg) is of great importance to the further investigation of the clinical relevance of these and other molecular genetic abnormalities in predicting the prognosis and clinical behavior of these tumors.

**Cytogenetics:**

- **Submit fresh, sterile tumor in sterile tissue transport media for cytogenetic analysis.**

Cytogenetic studies are valuable. Karyotyping of hepatoblastomas has revealed a recurrent pattern of chromosomal abnormalities. The most common karyotypic changes are extra copies of entire chromosomes (trisomies), sometimes in conjunction with other complex structural changes and often in association with double-minute chromosomes. Trisomies of chromosomes 2 and 20 have been most commonly reported. The clinical significance of trisomies is unknown at present, although a recent study using comparative genomic hybridization has suggested that chromosomal gains at chromosomes 8 and 20 may be associated with an adverse prognosis. A unique translocation has been reported in undifferentiated small cell hepatoblastoma, a variant associated with a poor prognosis, although this cytogenetic variant has not been reported in other cases.

**Immunohistochemistry:**

Immunohistochemistry with glypican-3, beta-catenin, and glutamine synthetase (GS) may help differentiate hepatoblastoma from normal liver or other hepatocellular tumors, or aid in accurate diagnosis of the various hepatoblastoma subtypes.

*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:

## HEPATOBLASTOMA (PEDIATRIC LIVER): Resection

- **Procedure:**

**Biopsy:**
- Core biopsy
- Incisional biopsy

**Notes:**
Biopsy types include image-guided needle biopsy or open biopsy. While it is easier to obtain tissue for studies with open biopsies, a needle biopsy performed in interventional radiology is adequate as long as 5-10 needle cores are obtained. It is recommended that the radiologist obtain needle cores from different portions of the tumor to maximize sampling of all areas of the tumor. The region from which the biopsy is obtained should be noted if possible. If tumor involves more than one lobe, more than one lesion or area of the tumor should be sampled. These sites should be labeled separately, as different nodules in the same patient may have different histology and biology. Use of intraoperative frozen sections should be discouraged unless the operative procedure will be altered by the result (education of the surgeon may be required).

**Biopsy Tumor Triage:**
- Every attempt should be made to assess if tissue obtained is viable and can be triaged for other studies.
    - Obtain touch preparations (imprints) for tumor submitted for cytologic examination.
    - Snap freeze tumor and nontumoral tissue.
    - Submit tissue in sterile tissue transport media for cytogenetic analysis.
    - Submit tissue in cold glutaraldehyde for electron microscopy.

**Resection:**
- Right lobectomy
- Extended right lobectomy
- Medial segmentectomy
- Left lateral segmentectomy
- Total left lobectomy
- Explanted liver
- Other (specify)

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

**Core Biopsy:**
- Length and diameter, or aggregate measurement if multiple cores

**Incisional Biopsy:**
- Measurement in three dimensions

**Hepatectomy, Lobectomy, Segmentectomy:**
- Weight

- Measurement in three dimensions
- If gallbladder is included, measure in three dimensions
- Include measurement of diaphragm if included

*Resectability is the key prognostic feature for all liver malignancies, with few exceptions. Unfortunately, 67% of reported cases are not amenable to primary surgery (Pediatric Oncology Group/Children's Oncology Group sample).*

- **Specimen Integrity and Adequacy:**

  - Specify if the liver capsule is intact or disrupted.
    - A disrupted capsule may indicate intraoperative tumor spill.

- **Tumor Triage:**

  - First priority should be given to formalin fixed tissue for morphologic evaluation.
  - Second priority for tissue processing is snap-freezing up to 1 gram (minimum 100 mg) of tumor from the grossly different regions for molecular studies.
  - Samples of non-tumoral liver should also be collected for snap-freezing as well.
  - Submit fresh, viable sterile tumor in sterile tissue transport media for cytogenetic studies.
  - Samples from the same foci should be collected for histology, with appropriate identification.

- **Photographic Map:**

  - A photographic map or diagram of the external and cut surface is beneficial to demonstrate the exact site from which each section is taken.
    - Document margins in lobectomy specimens.
    - Document hilar and vena caval margins in explants.
    - Document the site of biopsy or specific regions of tumor sampling, including sections from various nodules.

## CHILDREN'S ONCOLOGY GROUP STAGING

| | |
|---|---|
| Stage 1 | Tumors are completely resected, margins grossly and microscopically negative for tumor. |
| Stage II | Tumors are grossly resected with evidence of microscopic residual tumor. Such tumors are rare, and patients with this stage have not fared differently from those with stage I tumors in previous protocols. Resected tumors with preoperative (intraoperative) rupture are classified as stage II. |
| Stage III | (Unresectable) tumors are those that are considered by the attending surgeon not to be resectable without undue risk to the patient. These include partially resected tumors with measurable tumor left behind. They do not include grossly resected tumors with microscopic disease at the margins or resected tumors with preoperative/intraoperative rupture. Lymph node involvement is considered stage III disease and may require evaluation with second laparotomy after an initial 4 courses of chemotherapy. |
| Stage IV | Tumors that present with measurable metastatic disease to the lungs or other organ. *<br><br>*Nodal involvement of the inferior phrenic lymph nodes or other lymph nodes distal to the hilar, hepatoduodenal ligament, or caval lymph nodes are considered distant metastases. |

## TUMOR

- **Tumor Size:**
  - Specify tumor size in three dimensions for each tumor.
  - Specify weight for resection specimens.
  - For resection specimens, sections should be taken from each major/dominant tumor.
  - Submit representative sections from smaller tumors.
  - Submit at least one section per centimeter of tumor(s).

- **Tumor Focality (within liver):**
  - Unifocal
  - Multifocal

- **Tumor Site(s):**
  - Right lobe
  - Left lobe
  - Right and left lobes
  - Other (specify)

- **Tumor Extent of Involvement, Relationship to Attached Organs / Structures, Macroscopic Features, and Lymphovascular Invasion:**
  - **Macroscopic Extent of Tumor at Operation:**
    - Specify if tumor is confined to the liver.
    - Specify if tumor extends into adjacent organ(s).
    - Specify if tumor extends into adjacent soft tissue.
      - Diaphragm
      - Abdominal wall
      - Other (specify)
  - **Macroscopic Features:**
    - Identify any cysts, foci of bone, areas of necrosis, or hemorrhage. *

    *Necrosis and hemorrhage are frequently seen in hepatoblastomas. Tumor within the liver may have an extremely variegated appearance signifying the presence of mesenchymal tissue and epithelial cells. Various types of mesenchymal tissues (e.g., osteoid, cartilaginous, fibrous, muscle) in the mixed type of hepatoblastoma may alter the color and consistency of the gross appearance.

    Approximately 20% of the mixed type of hepatoblastomas contain a variety of tissues, including stratified squamous epithelium, melanin pigment, mucinous epithelium, cartilage, bone, and striated muscle. These tumors are designated as teratoid hepatoblastomas.

- **Lymphovascular Invasion: ***
    - Specify if there is macroscopic portal vein invasion by tumor.
    - Specify if there is macroscopic hepatic vein invasion by tumor.

**Documentation should include macroscopic vascular invasion versus intravascular growth found only microscopically, and whether it is present within tumor nodules or present in vessels of parenchyma outside of tumor nodules.*

- **Margins:**

**Resection Margins:**

- State if the surgical resection margins are macroscopically involved or uninvolved by tumor.
- Apply ink to the surgical resection margins.
- Judiciously sample the cut surface in the region closest to the nearest identified tumor nodule; specify the margin.
- In selected cases, adequate random sampling of the cut surface may be sufficient.
- If the neoplasm is found near the surgical resection margin, the distance from the margin should be reported in cm; specify the margin and section perpendicular to the lesion.
- For multiple tumors, the distance from the nearest tumor to the margin should be stated.
- Vascular margins:
    - Submit sections of the following vascular margins if macroscopically identified: *
        - Portal vein
        - Hepatic vein
        - Inferior vena cava

**Dissemination of hepatic malignancies occurs within portal veins and follows the expected ready access of infiltration into hepatic veins, with frequent lung involvement.*

- **Explanatory Notes:**

*The evaluation of margins for total or partial hepatectomy specimens depends on the method and extent of resection. It is recommended that the surgeon be consulted to determine the critical foci within the margins that require microscopic evaluation. The transection margin of a partial hepatectomy may be large, rendering it impractical for complete examination. In this setting, macroscopically positive margins should be microscopically confirmed and documented.*

**Capsular Surface:**

- **If macroscopically positive**, state and provide sections demonstrating the capsular surface where involved (penetrated) by tumor.
- **If macroscopically negative**, record the distance of invasive tumor from the closest capsular surface in cm, and provide sections showing the closest distance.
    - Specify the margin macroscopically involved.

- **Explanatory Note:**

  *Capsular rupture of subcapsular masses either before or during surgery can upstage an otherwise resectable malignancy. Consequently, even if tumor does not penetrate the liver capsule macroscopically, if tumor is exposed by a defect in the liver capsule this must be stated and sections showing the defect and exposed tumor must be submitted.*

## LYMPH NODES

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

  **Hepatic Regional Lymph Nodes Include:**

  - Hilar
  - Hepatoduodenal ligament
  - Caval

  **Histologic examination of a regional lymphadenectomy specimen usually involves examination of 3 or more lymph nodes:**

  - Count the number of lymph nodes identified.
  - Specify the location, if known.
  - Measure the lymph nodes in three dimensions.
  - Describe the cut surface of the 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.

## METASTASIS

- **Metastasis:**

  Dissemination of hepatic malignancies occurs within portal veins and follows the expected ready access of infiltration into hepatic veins, with frequent lung involvement. Further spread to the brain may occur. Hilar lymph node metastases are relatively infrequent, but capsular rupture of subcapsular masses either before or during surgery can upstage an otherwise resectable malignancy.

  **Distant Metastasis:**

  Distant metastasis includes nodal involvement of the inferior phrenic lymph nodes or other lymph nodes distal to the hilum, hepatoduodenal ligament, or caval region.

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