# Protocol for the Examination of Specimens from Patients with Hodgkin Lymphoma

**Protocol applies to Hodgkin lymphoma involving any site. This protocol does not apply to non-Hodgkin lymphoma or other lymphoid neoplasms. No TNM categories exist for Hodgkin lymphoma.**

**Based on:**

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**Revision History:**

None

**Summary of Changes:**

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

**Procedures Covered in this Protocol:**

- Biopsy
- Resection of Lymph Node(s) or Other Organ(s)

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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 and Immunohistochemistry Considerations:**

*Although the diagnosis of Hodgkin lymphoma does not per se require the use of molecular testing, flow cytometric analysis and cytogenetics, the use of such techniques might be necessary for diagnostic workup and distinction between Hodgkin lymphoma and non-Hodgkin lymphoma. Consequently, specimen triage is important. When tissue is adequate, one may consider the following tissue triage method and prioritization*:

- Tissue should be received fresh and cut into thin (2 mm) slices perpendicular to the long axis of the node.
    - Estimated time from excision to fixation should be noted, if possible, as this may impact preservation or recovery of certain analytes such as RNA and phosphoproteins in fixed tissues.
    - Touch imprints may be made from the freshly cut surface, and the imprints fixed in alcohol or air dried. Unstained air-dried imprints can be used for fluorescence in situ hybridization (FISH) or other studies if necessary.
- A generous portion of the sample should be submerged in 10% neutral buffered formalin for fixation.
    - Over-fixation (i.e., more than 24 hours) in formalin should be avoided for optimal immunophenotypic reactivity.
    - See note below related to immunophenotyping considerations.
- A portion of the sample should be submerged in an appropriate transport medium (such as RPMI) for flow cytometric analysis.
- If appropriate, a portion may be submitted for microbial cultivation.
- Portions may be fixed in zinc formalin or B5 fixative. These fixatives produce good cytologic detail but are not suitable for DNA extraction and may impair immunostains.
- Portions may be snap frozen and stored at -70 degrees C.

**Immunophenotyping (Immunocytochemistry) Considerations:**

Immunophenotyping is necessary in the initial diagnosis of Hodgkin lymphoma, and is useful in addition to histologic interpretation in determining if the background lymphocytic infiltrate is malignant by correlating antigen expression with architecture and cytology.

**Sample requirements for immunophenotyping:**

- Formalin-fixed paraffin-embedded (FFPE) tissue block **OR**
- Unstained slides (3-4 µm thick sections on positively charged slides; two to three slides per marker requested)
- A stained H&E slide (4-5 µm thick section)

**Hodgkin Lymphoma Immunophenotypes:**

- Nodular lymphocyte predominant Hodgkin lymphoma: Lymphocyte predominant cells (LP cells; previously called L&H cells) are CD20+, CD79a+, PAX5+, CD45+, BCL6+, OCT-2+, BOB.1+, EMA +/-, CD15-, CD30-, CD43-, EBER-.
- Nodular sclerosis classical Hodgkin lymphoma: Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+(weak), CD20-/+, CD79a-/+, EBER-/+, OCT-2-/+, BOB.1-/+, EMA-

- Mixed cellularity classical Hodgkin lymphoma: Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+(weak), CD20-/+, CD79a-/+, EBER+/-, OCT-2-/+, BOB.1-/+, EMA-
- Lymphocyte-rich classical Hodgkin lymphoma: Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+(weak), CD20-/+, CD79a-/+, EBER-/+, OCT-2-/+, BOB.1-/+, EMA-
- Lymphocyte-depleted classical Hodgkin lymphoma: Classical Hodgkin/Reed-Sternberg cells are CD30+, CD15+/-, CD45-, PAX5+(weak), CD20-/+, CD79a-/+, EBER+/-, OCT-2-/+, BOB.1-/+, EMA-

## PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

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

  - Biopsy
  - Resection
  - Other (specify)

  *This protocol does not apply to non-Hodgkin lymphoma or other lymphoid neoplasms.

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

  Many specimen types may be submitted for the evaluation of Hodgkin lymphoma. The most common specimen types include lymph nodes, mediastinal masses, bone marrow, spleen, lung, and liver.

  - Bone marrow biopsy – Provide length and diameter.
    - Bone marrow aspiration clot – Provide three dimensions.
  - Core biopsies of other tissues – Provide length and diameter.
  - Lymph node(s) excisional biopsy and resection – Provide measurement in three dimensions.
  - Mediastinal mass – provide measurement in three dimensions or an aggregate measurement if multiple fragments are submitted.
  - Splenectomy – provide weight and measurement in three dimensions or an aggregate measurement if submitted in the fragmented or morcellated state.
  - Any soft tissue resection – provide measurement in three dimensions.

  *As a result of improved diagnostic imaging techniques, staging laparotomy and pathologic staging have been abandoned.

  - The staging classification for lymphoma uses the term *lymph node region*. Lymph nodes are divided into core nodal regions:

    - Lymph nodes above the diaphragm:
      - Waldeyer's ring
      - Right/left cervical lymph nodes (cervical, supraclavicular, occipital, preauricular)
      - Right/left axillary
      - Right/left infraclavicular
      - Mediastinal
      - Right/left pulmonary hilar

    - Lymph nodes below the diaphragm:
      - Spleen
      - Para-aortic
      - Mesenteric
      - Right/left pelvic
      - Right/left inguinofemoral

- **Specimen Integrity, Adequacy, and Criteria for Organ Involvement:**

  - Excisional lymph node biopsy is the preferred specimen for diagnosis, subtyping, and grading of nodal malignant lymphoma to ensure representation of disease and avoidance of traumatic artifact.

  - Fresh lymphoid tissue should be examined by macroscopic inspection, touch preparations, or frozen section to assess adequacy of tissue for diagnosis.

  - Splenectomy capsular defects, disruption, or specimen fragmentation should be clarified with the surgeon and documented with indication as surgical defect, tumor disruption or traumatic defect. Documentation should include, if applicable, size (three dimensions), appearance (smooth, irregular, nodular, plaques), and the distance to the hilar margin. State if margin assessment cannot be made due to specimen fragmentation or disruption.

  - Organ Involvement:

    Bone marrow biopsies:
    - Bone marrow trephine (core) biopsies that fail to show lymphoma and consisting largely of periosteum, cortical bone, and subcortical marrow should not be considered adequate specimens. Repeat biopsies should be performed on the iliac crest bone.
    - Unilateral/bilateral bone marrow trephine (core) biopsies and aspirations should be submitted in children who present with B symptoms or advanced stage disease (III/IV).

    Lymph node(s) core biopsy or FNA:
    - Fine needle aspiration (FNA) on lymph node(s) should not be submitted for lymphoma studies, due to the high false-negative rate.

    Splenectomy:
    - Criteria for organ involvement include unequivocal palpable splenomegaly with multiple focal defects that are neither cystic nor vascular and demonstrated radiologically (ultrasound or CT), however, radiologic splenomegaly alone is insufficient.

    Soft tissue core biopsy and resection:
    - Criteria for organ involvement include clinical or radiologic imaging of large subcutaneous mass with or without evidence of nodal or skin involvement.

    Liver core biopsy:
    - Criteria for organ involvement include an enlarged liver with radiologic findings of multiple focal defects that are neither cystic nor vascular.
    - Abnormalities in liver function tests must be assessed as clinical enlargement of the liver alone is insufficient.

    Lung biopsy:
    - Criteria for organ involvement include radiologic involvement of parenchyma with alternative pathology, especially infection, ruled out. Biopsies may be required to clarify equivocal cases.

    CNS:
    - Criteria for organ involvement include spinal intradural deposits, spinal cord or meningeal involvement diagnosed on the basis of patient history and supporting radiologic findings (ultrasound, CT, MRI), and CSF examination.

- Spinal extradural deposits should be carefully assessed on a case-by-case basis, as they can be an extension of a soft tissue disease from bone metastasis or disseminated disease.
- Intracranial involvement is rarely diagnosed clinically at presentation, therefore, lymphoma should be considered on the basis of a space-occupying lesion in additional extranodal sites.

- **Specimen submission and triage considerations:**

  - Tissue should be received in the fresh state. Unsectioned lymph nodes should not be immersed in fixative.

  - Care should be taken to cut thin (2 mm) sections perpendicular to the long axis of the node to ensure optimal penetration of fixative.

  - Record the size, color, and consistency and any macroscopic presence of nodularity, hemorrhage, or necrosis of the lymph node.

  - If possible, estimate time from excision to fixation as this may impact preservation or recovery of certain analytes such as RNA and phosphoproteins in fixed tissues.

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

  - *A generous 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.*

  - *A portion of the sample should be submerged in an appropriate transport medium (such as RPMI) for flow cytometric analysis.*
    - *As mentioned, flow cytometry does not assist in acquiring a diagnosis of Hodgkin lymphoma, but is very important in establishing a diagnosis of non-Hodgkin lymphoma. Thus, if the sample is adequate, and the diagnosis uncertain, it is preferable to triage the sample with relatively high priority given to flow cytometric analysis.*

  - *If appropriate, a portion may be submitted for microbial cultivation.*

  - *Portions may be fixed in zinc formalin or B5. These fixatives produce superior cytologic detail but are not suitable for DNA extraction and may impair immunostains.*
    - *Take steps to ensure that the macroscopic description indicates the fixative used for each cassette submitted.*

  - *Portions may be snap frozen and stored at -70 degrees C.*

## TUMOR

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

- **Tumor Size:**

  - Bone marrow biopsies and aspirates, core biopsies (lymph node(s), soft tissue, liver, lung) and CNS fluid:
    - Record appropriate measurements; **OR**
    - Unable to determine dimensions macroscopically.

  - Lymph node(s) and soft tissue for excisional biopsy or resection:
    - Record measurements in three dimensions.
    - State if tumor has a unifocal solitary appearance; **OR**
    - Unable to determine dimensions macroscopically.

  - Splenectomy:
    - Solitary nodule - Record three dimensions.
    - Multiple nodules - State number of nodules present and provide a range in size in three dimensions.

- **Tumor Site(s):**

  Hodgkin lymphoma is almost always nodal based with cervical lymph nodes more commonly involved. Mediastinal, axillary, and paraaortic lymph node sites are also frequently involved. Extranodal Hodgkin lymphoma is rarely seen.

  - Specify site of lymph node(s), if identified.
  - Specify other tissue(s) or organs(s) submitted.

    - Lymph node(s):
      - Nodes larger than 1.5 cm are considered abnormal.
      - Involved lymph node(s), soft tissue and splenic nodules typically have a homogeneous tan/white fleshy appearance.

    - Bone Marrow:
      - Unusual for Hodgkin lymphoma of any subtype to present solely as a bone marrow infiltration at initial presentation, however, it is relatively common to be present at relapse.

## PATHOLOGIC STAGING

Staging involves a combination of clinical, radiologic and surgical data. Imaging, biopsy, and bone marrow examination are often the main criteria for staging Hodgkin lymphoma.

- **AJCC Prognostic Stage Groups**

**Lugano Classification for Hodgkin and Non-Hodgkin Lymphoma**

| Stage | Stage Description |
|---|---|
| ***Limited Stage*** | |
| I | Involvement of a single lymphatic site (i.e., nodal region, Waldeyer's ring, thymus, or spleen) |
| IE | Single extralymphatic site in the absence of nodal involvement (rare in Hodgkin lymphoma) |
| II | Involvement of two or more lymph node regions on the same side of the diaphragm |
| IIE | Contiguous extralymphatic extension from a nodal site with or without involvement of other lymph node regions on the same side of the diaphragm |
| *II bulky** | *Stage II with disease bulk * * |
| ***Advanced Stage*** | |
| III | Involvement of lymph node regions on both sides of the diaphragm; nodes above the diaphragm with spleen involvement |
| IV | Diffuse or disseminated involvement of one or more extralymphatic organs, with or without associated lymph node involvement;<br>or *noncontiguous* extralymphatic organ involvement in conjunction with nodal stage II disease<br>or *any* extralymphatic organ involvement in nodal Stage III disease<br>Stage IV includes *any* involvement of the CSF, bone marrow, liver, or multiple lung lesions (other than by direct extension in Stage IIE disease) |

***Stage II bulky may be considered either early or advanced stage based on lymphoma histology and prognostic factors (see discussion of Hodgkin lymphoma prognostic factors).*

***The definition of disease bulk varies according to lymphoma histology. In the Lugano classification, bulk in Hodgkin lymphoma is defined as a mass greater than one third of the thoracic diameter on CT of the chest or a mass >10 cm. For NHL, the recommended definitions of bulk vary by lymphoma histology. In follicular lymphoma, 6 cm has been suggested based on the Follicular Lymphoma International Prognostic Index-2 (FLIPI-2) and its validation. In DLBCL, cutoffs ranging from 5 to 10 cm have been used, although 10 cm is recommended.*
*Note: Hodgkin lymphoma uses A and B designation with stage group. A/B is no longer used in NHL.*



- **Explanatory Notes:**

  - The "E" indicator designates either a single extranodal involvement, or a contiguous or proximal known nodal site of disease.

  - The "S" indicator designates splenic involvement.

- Lymph nodes above the diaphragm: Waldeyer's ring, cervical, supraclavicular, occipital, pre-auricular, infraclavicular, axillary, pectoral, mediastinal, hilar, epitrochlear, and brachial.

- Lymph nodes below the diaphragm: Splenic, mesenteric, para-aortic, iliac, inguinal, femoral, and popliteal.

- Stage IE is rare in Hodgkin lymphoma.

- *Stage IV includes any involvement with the following sites: liver, bone marrow, cerebrospinal fluid, and lungs (other than direct extension from another site).

## HISTOLOGIC TYPE (2008 WHO classification)

Universal, consensual world collaboration on the classification of Hodgkin lymphoma, based on morphological diagnostic criteria, clinical, and prognostic relevance that distinguishes between the rare variant from the four classical subtypes.

- **Types:**

  Hodgkin lymphoma, histologic subtype cannot be determined.
  Nodular lymphocyte predominant Hodgkin lymphoma.
  Classical Hodgkin lymphoma, histologic subtype cannot be determined.
  Classical Hodgkin lymphoma, subtype:
  - Nodular sclerosis classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Lymphocyte-depleted classical Hodgkin lymphoma

## Clinical Prognostic Factors and Indices:

- **A and B Classification (Symptoms):**

  Each Hodgkin lymphoma stage is classified as A (symptoms absent) or B (symptoms present) according the following defined constitutional symptoms: The Lugano classification includes B symptoms as a prognostic factor in Hodgkin lymphoma.

  **A (symptoms absent) and B (symptoms present) must be recorded to determine stage.**

  **B Symptoms**
  - Presence of Fever (>38°C, frequently in Pel-Ebstein pattern)
  - Drenching night sweats (e.g., those that require change of bedclothes)
  - Weight Loss (>10% of baseline within 6 months)

  The presence of B symptoms has a correlation with extent of disease (stage and tumor bulk), but symptoms have also been shown to have prognostic significance for cause-specific survival that is independent of stage.

- International Prognostic Score predicts outcome based on the following adverse factors:
  - Serum albumin <4g/dL
  - Hemoglobin concentration <10.5 g/dL
  - Male sex
  - Age >/= 45 years
  - Stage IV disease
  - White blood cell count >/= 15,000/$mm^3$
  - Lymphopenia <600/$mm^3$ or <8%

  Rate of Freedom from Progression by risk category:
  - 0 factors 84%
  - 1 factor 77%
  - 2 factors 67%
  - 3 factors 60%
  - 4 factors 51%
  - 5 or more factors 42%

REFERENCE REVIEW:

1. Amin, MB, Edge SB, Greene FL, Byrd DR, et al. (Eds.) *AJCC Cancer Staging Manual,* 8th ed. New York; NY: Springer; 2017.

2. Hussong JW, Arber DA, Bradley KT, et al. Protocol for the Examination of Specimens from Patients with Hodgkin Lymphoma. *CAP Cancer Protocol Hodgkin Lymphoma 3.1.0.1*. 2013.

3. Hussong JW, Arber, DA, Bradley KT, et al. Protocol for the Examination of Specimens from Patients with Hematopoietic Neoplasms involving the Bone Marrow. *CAP Cancer Protocol 3.0.1.1*. 2012.

4. Foucar K & Reichard K. *Bone marrow pathology*, 3rd ed., Vol. 2. Chicago: ASCP Press; 2010.

5. Rywlin AM. *Histopathology of the bone marrow*. Boston: Little, Brown; 1976.

6. Hematology/Oncology Clinics of North America. *Bone Marrow Examination*. Vol. 2/Number 4. W.B. Saunders Company, Harcourt Brace Jovanovich, Inc. December 1988.

7. Jaffe ES. *Hematopathology*. Philadelphia, PA: Saunders/Elsevier; 2011.

8. The Illustrated Guide to Bone Marrow Diagnosis from DAKO: Chapter 25 Hodgkin Lymphoma. DAKO. Retrieved from: http://www.dako.com/00164_11jun09_guide_to_bone_marrow_diagnosis chapter_25.pdf.

9. Kumar V, Abbas AK, Fausto N, Robbins SL, & Cotran RS. *Robbins and Cotran pathologic basis of disease*, 9th ed. Philadelphia: Elsevier Saunders; 2014.