Protocol for the Examination of Specimens from Patients with Non-Hodgkin Lymphoma/Lymphoid Neoplasms

Protocol applies to non-Hodgkin lymphoma/lymphoid neoplasms involving any site. This protocol does not apply to ocular adnexal lymphoma, lymphoid neoplasms of bone marrow, mycosis fungoides, and Sezary syndrome.

Based on:

AJCC/UICC TNM, 8th edition
CAP Cancer Protocol version: NonHodgkin 3.2.0.1
CAP Protocol Web Posting Date: October 2013
AAPA Macroscopic Examination Template Version 2.0
AAPA Web Posting Date: October 2018

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 Considerations and Specimen Triage:

Molecular testing, flow cytometric analysis, and cytogenetics are necessary for differential diagnosis of non-Hodgkin lymphoma however, these tests will not assist in diagnosis of Hodgkin lymphoma. Moreover, composite lymphomas and transformation from non-Hodgkin to Hodgkin lymphoma may pose diagnostic challenges.

Consequently, specimen triage is important. When tissue is adequate, one may consider the following tissue triage methods 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 95% 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 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.
    - As mentioned, flow cytometric analysis 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 fresh for microbial cultivation and in viral culture media for viral cultivation.

- Portions may be fixed in zinc formalin, B5 or B+. These fixatives produce superior cytologic detail but are not suitable for DNA extraction and may impair immunostains.

- Portions may be snap frozen and stored at -70 degrees C for tissue banking and clinical trials.

- Portions may be submitted sterile or in appropriate medium for cytogenetic analysis.

Immunophenotyping (Immunocytochemistry) Considerations:

Immunophenotyping is necessary in the initial diagnosis of non-Hodgkin lymphoma and can be performed by flow cytometry and/or immunohistochemistry.

Refer to the CAP protocol for a comprehensive listing of the recommended types of immunohistochemistry tests to consider depending on the type of non-Hodgkin lymphoma.

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)

As stated above, 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
  - Resection
  - Other (specify)

  * This protocol does not apply to ocular adnexal lymphoma, lymphoid neoplasms of bone marrow, mycosis fungoides, and Sezary syndrome.

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

  Many specimen types may be submitted for the evaluation of lymphoid neoplasms. The most common specimen types include lymph nodes, skin, gastrointestinal tract, bone marrow, spleen, thymus, and tonsils.

  - Lymph node(s) excisional biopsy and resection – Provide measurement in three dimensions.
  - Skin – Provide measurement in three dimensions.
  - Bone marrow biopsy – Provide length and diameter.
    - Bone marrow aspiration clot – Provide three dimensions.
  - Spleen – provide weight and measurement in three dimensions or an aggregate measurement if submitted in the fragmented or morcellated state.
  - Tonsils – Provide weight and three dimensions of each tonsil.
  - Thymus – Provide weight and three dimensions.
  - Gastrointestinal Tract – Provide three dimensions or length and diameter if the specimen is a bowel resection.
  - Gastrointestinal Tract endoscopic biopsies – Provide greatest dimension or aggregate dimensions.
  - Soft tissue resection – provide three dimensions.

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

- **Lymph Node Regions**

  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 (palatine, lingual, and pharyngeal tonsils)
    - 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:
    - Splenic

- Para-aortic
- Mesenteric
- Right / left pelvic
- Right / left inguinofemoral

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

  - Excisional lymph node biopsy provides ample material 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/imprints, 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 of tumor to the hilar margin. State if the margin assessment cannot be made due to specimen fragmentation or disruption.

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

  - 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) material may be used for flow cytometry immunophenotyping in addition to morphologic evaluation. On occasion, cell block preparations might be useful for immunohistochemistry immunophenotyping.

    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 Handling and Triage Recommendations:**

  - Excisional or incisional biopsy samples should be received in the fresh state on saline-moistened Telfa. Unsectioned lymph nodes should not be immersed in fixative.
  - Care should be taken to cut thin (2 mm) sections perpendicular to the long axis to the node to ensure optimal penetration of fixative.
  - Record the size, color, and consistency, and any macroscopic presence of nodularity, hemorrhage, or necrosis of 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 95% 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.
    - Flow cytometry 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, B5, or B+, although such a practice is not required and has been largely abandoned in most labs. These fixatives produce good 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.

- Portions may be submitted sterile or in appropriate medium for cytogenetic analysis.

TUMOR

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

- **Tumor Size:**
  - Bone marrow biopsies and aspirates, core biopsies, skin, gastrointestinal specimens:
    - Record appropriate individual measurements; OR
    - Provide aggregate macroscopic dimensions.
  - Lymph node(s) for excisional biopsy or resection:
    - Record measurement in three dimensions.
    - State if tumor has a unifocal or multifocal appearance.
  - Organs - Spleen, Tonsils, Thymus
    - Solitary nodules – record measurement in three dimensions.
    - Multiple nodules – State number of nodules present and provide a range in size in three dimensions.
  - Gastrointestinal Tract – Provide three dimensions of solitary nodule or range in size in three dimensions if multiple.

- **Tumor Site(s): ***
  - Specify site of lymph node, if identified.
    - Specify the color, consistency, and identify any areas of necrosis.
      - Lymphomas cause diffuse expansion of the node, so that it often appears rounded and loses the usual bean-shaped contour.
      - Lymphomas usually have a tan-white, fleshy appearance.
  - Specify site of other tissue(s) or organ(s) submitted.
  - Major structures of the lymphatic system:
    - Groups and chains of lymph nodes
    - Spleen
    - Thymus
    - Waldeyer's ring
    - Vermiform appendix
    - Peyer's patches of the ileum
  - Minor sites of lymphoid tissue:
    - Bone marrow
    - Mediastinum
    - Liver
    - Skin
    - Lung
    - Pleura
    - Gonads

*Involvement of lymphoma in extranodal sites is more common in non-Hodgkin lymphoma than in Hodgkin lymphoma.

- **Pathologic Extent of Tumor (Stage):**

  - Specify if there is macroscopic evidence of extracapsular spread into adjacent soft tissues or organ parenchyma. *
  - Specify site of involvement.

*Direct spread of a lymphoma into adjacent tissues or organs does not influence classification of stage.

Staging of non-Hodgkin lymphoma is currently and more commonly clinical rather than pathologic. Clinical staging involves a combination of clinical, radiologic, and surgical data. Physical examination, laboratory tests, imaging studies, biopsy, and bone marrow examination are required.

- **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; or *noncontiguous* extralymphatic organ involvement in conjunction with nodal stage II disease or *any* extralymphatic organ involvement in nodal Stage III disease Stage IV includes *any* involvement of the 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 extralymphatic involvement; a contiguous or proximal known nodal site of disease.
  - The “S” indicator designates splenic involvement.
  - Lymph nodes above the diaphragm include: Waldeyer’s ring, cervical, supraclavicular, occipital, preauricular, infraclavicular, axillary, pectoral, mediastinal, hilar, epitrochlear, and brachial.
  - Lymph nodes below the diaphragm include: splenic, mesenteric, para-aortic, iliac, inguinal, femoral, and popliteal.
  - Multifocal involvement of a single extralymphatic organ is classified as stage IE and not stage IV.
  - For all stages, tumor bulk greater than 10 to 15 cm is an unfavorable prognostic factor.
  - For stages II to IV, involvement of more than two sites is an unfavorable prognostic factor.
    For stages III to IV, a large mediastinal mass is an unfavorable prognostic factor.

HISTOLOGIC TYPE (2008 WHO classification)

Assignment of histologic type is based on the World Health Organization (WHO) classification of lymphoid neoplasms. This classification provides distinction of individual lymphoid neoplasms based on morphologic, immunophenotypic, cytogenetic, and clinical features.

Besides histologic examination, the majority of lymphoid neoplasms will require the employment of one or more ancillary techniques, such as immunophenotyping, molecular studies, and / or cytogenetic analysis to arrive at the correct diagnosis.

Clinical Prognostic Factors and Indices:

The Lugano classification includes B symptoms as a prognostic factor only in Hodgkin lymphoma.

A and B Classification (Symptoms):

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 Index for non-Hodgkin Lymphoma

- Age < or = 60 years of age vs > 60 years of age
- Tumor stage I or II vs III or IV
- ECOG performance status 0 or 1 vs 2 to 4
- Serum lactate dehydrogenase concentration normal vs above normal
- Number of extanodal sites of involvement 0 or 1 vs more than one extranodal site

Based on the number of risk factors, patients are assigned to 1 of 4 risk groups:

- Low (0-1 risk factor)
- Low intermediate (2 risk factors)
- High intermediate (3 risk factors)
- High (4 or 5 risk factors)

Freedom from Progression Rate by Risk Category

- Low risk – 87% complete response, 73% overall survival rate at 5 years
- High risk – 44 % complete response, 26% overall survival rate at 5 years

Note: Prognostic indices exist or are under development for other lymphoid neoplasms.

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