Protocol for the Examination of Specimens from Patients with Melanoma of the Skin

Protocol applies to melanoma of cutaneous surfaces only.

Based on:

AJCC/UICC TNM, 8th edition
CAP Cancer Protocol version: Melanoma 4.0.0.1
CAP Protocol Web Posting Date: June 2017
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 and the current version of the CAP Cancer Protocol Melanoma 4.0.0.1.

Procedures Covered in this Protocol:

- Biopsy (punch, shave, incisional)
- Excision
- Re-excision
- Lymphadenectomy (sentinel nodes, regional nodes)

Authors:

Brooke Walsh, MS, PA(ASCP)CM*
Morristown Pathology Associates, Morristown, NJ
Courtney Hyland, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Darryl Kinnear, PA(ASCP)CM
Department of Pathology, Baylor College of Medicine, Houston, TX
John Lehman, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Stephanie Miller, PA(ASCP)CM
Providence Health & Services, Portland, OR
Tina Rader, PA(ASCP)CM
Drexel University College of Medicine, Philadelphia, PA
Erica Reed, PA(ASCP)CM
Mayo Clinic, Rochester, MN
Mike Sovocool, MHS, PA(ASCP)CM
Pathology Associates of Syracuse, Syracuse, NY
Dennis Strenk, PA(ASCP)CM
Wisconsin Diagnostic Laboratories, Milwaukee, WI
Connie Thorpe, PA(ASCP)CM
Department of Pathology, Saint Louis University, St. Louis, MO
Jon Wagner, PA(ASCP)CM
Department of Pathology, Sutter Roseville Medical Center, Roseville, CA

*Denotes primary author. All other contributing authors are listed alphabetically.

Previous Lead Contributors:
Jennifer Wood, PA(ASCP)[CM]
Department of Pathology, University of Virginia, Charlottesville, VA

Art Director | Illustrator Liaison:
Jesse McCoy, BFA, MHS, PA(ASCP)[CM]
Hampton Roads Pathology, Chesapeake Regional Medical Center, Chesapeake, VA

Illustrator:
Tami Tolpa

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:

Studies suggest that *BRAF* mutations may be acquired during metastasis, and a definitive link between prognosis and *BRAF* mutations has not been observed. In the presence of metastasis, vemurafenib may be used to treat metastatic melanoma cases with *BRAF* V600E mutation.

NRAS mutations occur in 20% of melanomas, however, the clinical significance of NRAS inhibitors is still being investigated. KIT mutations occur in fewer that 5% of melanomas. KIT inhibitors are currently in clinical trials.

Immunohistochemical Considerations:

S100 or melan-A immunostains may help highlight malignant melanoma cells in the dermis that might otherwise be obscured by a heavy lymphocytic infiltrate at the base of a lesion.

Some malignant melanomas are cytokeratin (CAM 5.2), CEA, and EMA positive. Ki-67 proliferation index and p53 are increased in malignant melanocytic lesions. Ki-67 index is a more accurate prognostic indicator in established malignant melanoma than mitotic count.

The use of immunohistochemical stains (e.g., for HMB-45 or MART-1) increases the sensitivity of detection of microscopic melanoma metastases and should also be considered in the examination of sentinel lymph nodes.

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 (punch, shave, other)
  - Excision (shave removal, saucerization, ellipse, wide, other)
  - Re-excision (ellipse, wide, other)
  - Lymphadenectomy (sentinel nodes, regional nodes)

- **Explanatory Notes:**

  Optimal evaluation of melanocytic lesions requires complete excision that incorporates the full thickness and lateral border of the involved lesion removed intact, therefore shave and punch biopsy procedures that may not include intact base and lateral borders should be avoided.

  Frozen sections in biopsies or excisions are highly discouraged; optimal histological evaluation requires well cut and stained sections prepared from formalin fixed paraffin embedded tissue.

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

  - Biopsy: measure in three dimensions, length x width x depth.
  - Excision (shave removal, saucerization, ellipse, wide, other): measure skin surface in two dimensions and maximum depth.
  - Re-excision (ellipse, wide, other): measure skin surface in two dimensions and maximum depth.
  - Lymphadenectomy:
    - Sentinel – measure each node in three dimensions.
    - Regional – measure each node in three dimensions, if multiple, provide a range in size.
    - Provide comment on the size of macroscopically positive nodes.

- **Specimen Laterality:**

  - Specify specimen laterality:
    - Right
    - Left
    - Midline

- **Specimen Integrity and Adequacy:**

  Note if defects to the margin or epithelium are present. Describe the anatomic location of the defect from the tumor and relevant margins. If defects involve the tumor and serve to hinder assessment of the final surgical margin, consultation with the surgeon for clarification should be considered, as well as differentially inking the margins in areas affected by defects as this may assist in creating a post-surgical treatment plan.

TUMOR ("T of TNM)

- **Tumor Size**:

  - Provide three dimensions of the tumor (s), length x width x depth.
  - If a re-excision, with scar but no gross residual tumor, provide the same measurements; distinguishing that the measurements apply to a scar with no residual tumor seen.

- **Tumor Site(s):**

  - Specify tumor site, (if known).
    - For cutaneous melanoma, prognosis may be affected by primary anatomic site.
  - State if unifocal or multifocal.
  - Satellite and in-transit metastases affect staging adversely and have a biologic impact that is comparable to that of nodal metastases.

    - Satellite metastasis is characterized by the presence of tumor nests or nodules (macroscopic or microscopic) within 2 cm of the primary melanoma but separated from the main tumor nodule by uninvolved dermis and / or subcutis.

    - An in-transit metastasis is a focus of metastatic melanoma in the skin or subcutaneous tissue more than 2 cm from the primary melanoma but not beyond the first draining lymph node basin.

- **Macroscopic Appearance and Pigmentation:**

  - Verrucous
  - Nodular
  - Sessile
  - Ulcerated
  - Pigmented or non-pigmented
    - Pigmentation not identified
    - Focal/patchy
    - Diffuse
  - Halo
  - Satellite lesions
  - Scarring

- **Tumor Depth of Invasion and Relationship to Attached Organs/Structures:** (*Figure 1*)

  - Measure the thickness of the tumor.
    - The upper reference point is the top of the epidermis of the overlying skin or, if the lesion is ulcerated, the base of the ulcer.
    - The lower reference point is the deepest point of tumor invasion, excluding tumor surrounding skin appendages.

- Indicate if the tumor is confined to the epidermis, extends into the subcutaneous tissue, or transects at the deep resection margin.

- Describe if the tumor invades into attached underlying organs / structures, (mucosa, muscle, cartilage or bone).

- Submit sections showing the deepest point of invasion, making sure to take sections perpendicular to the skin surface so that the microscopic measurement of tumor thickness is precise.

Breslow thickness is an important prognostic parameter associated with invasive melanoma.

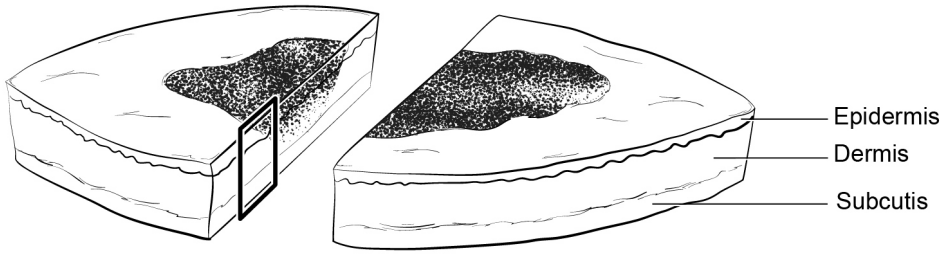
- Measurements of the clinical lesion (width and breadth) together with measured Breslow thickness provide an estimate of tumor volume.
    - An optical micrometer is aligned perpendicularly to the epidermis, and the melanoma is measured from the top of the granular cell layer or ulcerated tumor surface to the deepest point of invasion, avoiding the adventitial dermis along adnexal structures.
    - If the tumor is transected by the deep margin of the specimen, the depth may be indicated as “at least __mm” with a comment explaining the limitation of thickness assessment.

Clark Levels

I Intraepidermal tumor only
II Tumor present in but does not fill and expand papillary dermis
III Tumor fills and expands papillary dermis
IV Tumor invades into reticular dermis
V Tumor invades subcutis

- **Explanatory Note:**

Although Clark level of invasion has prognostic significance, many publications have demonstrated that the level of invasion is less reproducible among pathologists and does not reflect prognosis as accurately as tumor thickness. Clark levels were previously required for subclassifying pT1 lesions according the AJCC 6th Edition TNM classification system and were commonly reported. Anatomic level was replaced by mitotic rate in the AJCC 7th Edition tables for subclassifying pT1 lesions as T1a or T1b, but in the AJCC chapter, Clark level IV or V was referred to as a tertiary criterion for T1b in cases with no ulceration and “if mitotic rate cannot be determined.” Clark level is not used in the 8th Edition staging system but should be recorded as a primary tumor characteristic.



Clark Level and Breslow Thickness

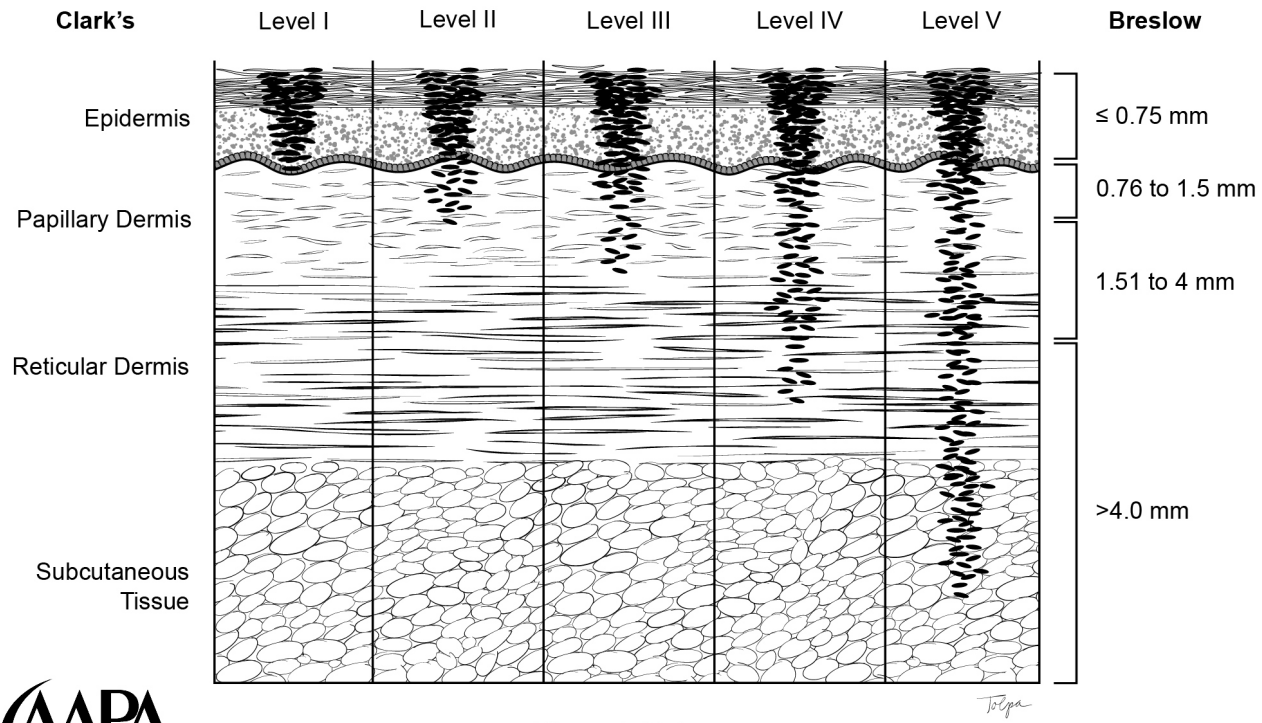


Figure 1: Melanoma

Clark Levels:
Level I: Confined to the epidermis
Level II: Invasion of the papillary (upper) dermis
Level III: Filling of the papillary dermis, but no extension into the reticular (lower) dermis
Level IV: Invasion of the reticular dermis
Level V: Invasion of the deep subcutaneous tissue

- **Ulceration:**

  - The presence or absence of ulceration should be documented in the macroscopic description.
    - The presence of ulceration modifies the pT stage of a melanoma from subcategory a (non-ulcerated) to b (ulcerated).
  - Ulcerated melanomas typically show invasion through the epidermis, whereas non-ulcerated melanomas tend to lift the overlying epidermis.
  - There is a correlation between ulceration and thickness.
    - For ulcerated tumors, the median thickness is about 3 mm.
    - For nonulcerated tumors, the median thickness is about 1.3 mm.
  - Post traumatic ulceration should be distinguished from ulceration due to invasion by tumor when possible. The width of the ulcerated area should be reported.

- **Explanatory Notes:**

  - Ulceration is a dominant prognostic factor in cutaneous melanoma without metastasis. A significantly worse prognosis and a higher risk of metastatic disease have been demonstrated for ulcerated versus non-ulcerated tumors of equivalent thickness.

  - Superficial spreading melanomas demonstrate prominent radial growth and have a better prognosis than nodular melanomas, which predominately demonstrate vertical growth.

Definition of Primary Tumor (pT)

pT Category	Thickness	Ulceration Status
pTX: primary tumor cannot be assessed (e.g., diagnosis by curettage)	Not applicable	Not applicable
mmpT0: no evidence of primary tumor (e.g., unknown primary or completely regressed melanoma)	Not applicable	Not applicable
pTis (melanoma *in situ*)	Not applicable	Not applicable
pT1	≤ 1mm	Unknown or unspecified
pT1a	< 0.8 mm	Without ulceration
pT1b	< 0.8 mm 0.8 – 1 mm	With Ulceration With or without ulceration
pT2	>1.0 - 2.0 mm	Unknown or unspecified
pT2a	>1.0 - 2.0 mm	Without ulceration
pT2b	>1.0 - 2.0 mm	With Ulceration
pT3	>2.0 – 4 mm	Unknown or unspecified
pT3a	>2.0 – 4 mm	Without ulceration
pT3b	>2.0 – 4 mm	With ulceration
pT4	>4 mm	Unknown or unspecified
pT4a	>4 mm	Without ulceration
pT4b	>4 mm	With ulceration

***TNM Descriptors**:*

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

Post-therapy stage (yTNM) documents the extent of the disease for patients whose first course of therapy includes systemic or radiation treatment prior to surgical resection or when systemic therapy or radiation is primary treatment with no surgical resection. The extent of disease is classified using the same T, N, and M definitions and identified as post-treatment with a "yc" or "yp" prefix (ycT, ycN, ycTNM; ypT, ypN, ypTNM).

Retreatment classification (rTNM) is used because information gleaned from therapeutic procedures and from extent of disease defined clinically may be prognostic for patients with recurrent cancer after a disease-free interval. It is important to understand that the rTNM classification does not change the original clinical or pathologic staging of the case.

Autopsy classification (aTNM) is used to stage cases of cancer not recognized during life and only recognized postmortem.

Residual Tumor (R)

The absence or presence of residual tumor at the primary tumor site after treatment is denoted by the symbol R. The R categories for the primary tumor site are as follows:

R	R Definition
RX	Presence of residual tumor cannot be assessed
R0	No residual tumor
R1	Microscopic residual tumor
R2	Macroscopic residual tumor at the primary cancer site or regional nodal sites (This designation is not used to indicate metastatic disease identified but not resected at surgical exploration.)

For the surgeon, the R classification may be useful to indicate the known or assumed status of the completeness of a surgical excision. For the pathologist, the R classification is relevant to the status of the margins of a surgical resection specimen. That is, tumor involving the resection margin on pathologic examination may be assumed to correspond to residual tumor in the patient and may be classified as macroscopic or microscopic according to the findings at the specimen margin(s).

- **Margins:**

  If an orienting suture is present, use the clinical designation. Differentially apply ink to the surgical resection margins.

  - **Peripheral margins:**
    - **If uninvolved**: state if the margin is macroscopically uninvolved by tumor or scar.
      - Record the distance of tumor or scar from the closest peripheral margin in mm (excisions only).
      - Specify the location, if possible.
      - Submit sections showing the relationship of the tumor or scar to the margin.
      - If the margin is widely free, such that the distance between the tumor or scar and margin is beyond what can be shown on a slide, the tumor or scar may be removed from the remainder of the excision and entirely submitted with a rim of surrounding normal tissue. Following this, the margin may be submitted as shave sections (to rule out the possibility of microsatellites, satellites or in transit metastasis at the margins. In addition, any tissue not submitted for microscopic evaluation must be sectioned at narrow intervals and inspected for the presence of satellites / in transit metastasis.
    - **If involved**: state if the margin is macroscopically involved by tumor or scar.
      - Specify the location, if possible.
      - Submit sections showing the tumor or scar extending to the involved margin.
      - Submit sections showing other, uninvolved margins, as described above.

  - Deep margin:
    - **If uninvolved**: state if the margin is uninvolved by tumor.
      - State the distance of tumor from the margin in mm (excisions only).
      - Submit sections showing the relationship of the tumor to the deep margin.
    - **If Involved**: state if the margin is involved by tumor.
      - Submit sections showing the tumor extending to the involved deep margin.

- **Explanatory notes:**

  - A “safe minimum” margin has not been established in the literature. CAP Protocol for the Examination of Specimens From Patients With Melanoma of the Skin encourages reporting the distance of invasive melanoma and melanoma in situ from the closest margins. When handling a large re-excision, it may not be necessary to submit the entire excision. However, after consultation with the attending pathologist, the macroscopic processing steps should include:

    - Submission of the entire tumor or scar including a rim of surrounding normal tissue.
    - If macroscopically uninvolved, full thickness submission of the entire peripheral margin (if feasible) to rule out melanoma in situ, or satellites / in transit metastasis at the margin.
    - Carefully examine all tissue that is not submitted for microscopic examination to rule out the possibility of macroscopic satellites / in transit metastasis with definite statements either to the negative (“no satellites or in transit metastasis seen”) or positive (positive statements must be accompanied by providing the distance of the satellite or in transit metastasis both from the primary tumor and

nearest margin; and, sections demonstrating the satellite / in transit metastasis and nearest margin must be provided).

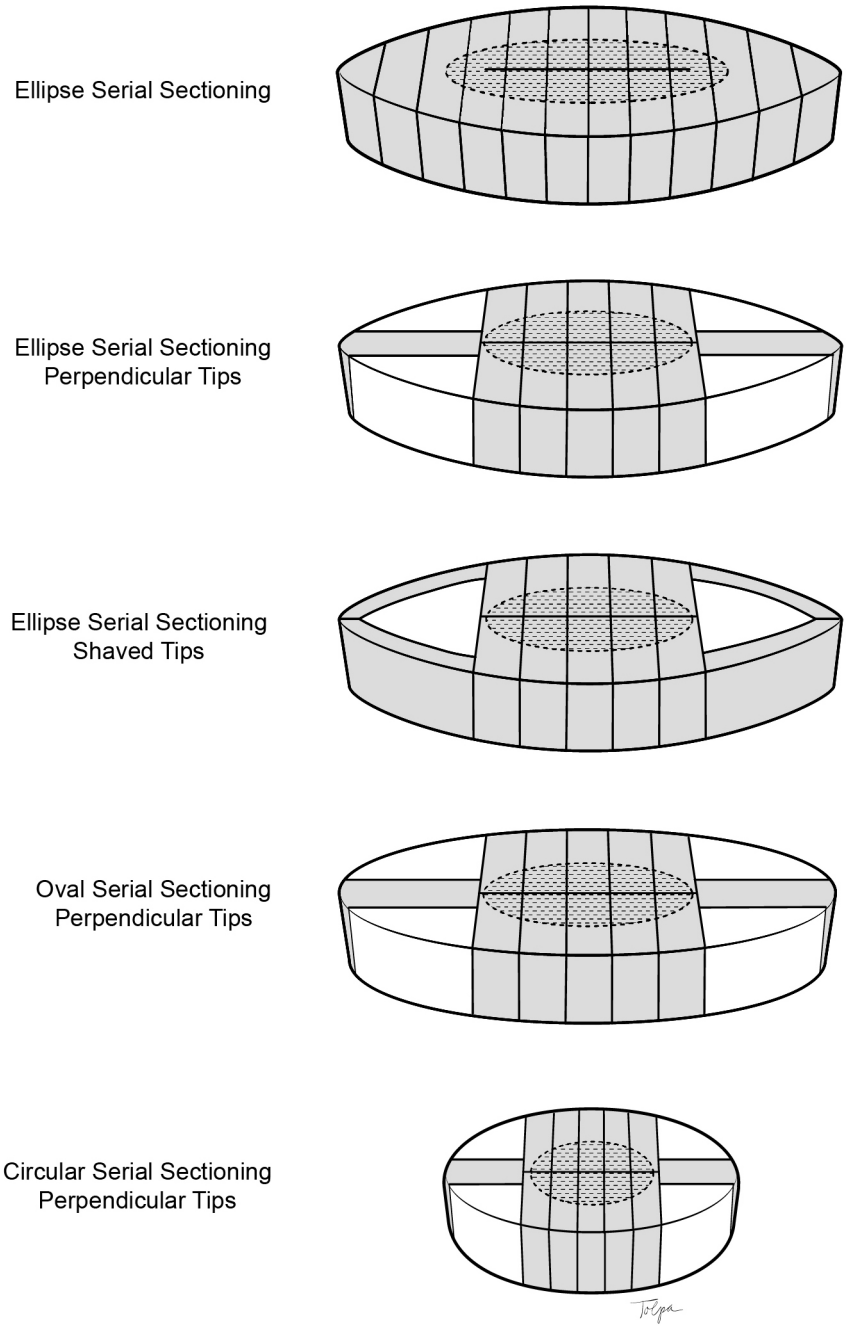


Figure 2: Melanoma Sectioning Techniques

LYMPH NODES ("N" of TNM)

- **Lymph Nodes:** (applicable to invasive tumor only)

Definition of Regional Lymph Node (pN)

pN Category	Number of tumor-involved regional lymph node	Presence or in-transit, satellite, and/or microsatellite metastasis
pNX	Regional lymph nodes cannot be assessed (e.g., SLN biopsy not performed, regional nodes previously removed for another reason) Exception: pathological N category is not required for T1 melanomas, use cN.	No
pN0	No regional lymph node metastasis	No
pN1	One tumor-involved node or in-transit, satellite, and/or microsatellite metastases with no tumor involved nodes	
pN1a	One clinically occult (i.e., detected by SLN biopsy)	No
pN1b	One clinically detected tumor-involved node	No
pN1c	No regional lymph node disease	Yes
pN2	Two or three tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with one tumor-involved node	
pN2a	Two or three clinically occult tumor-involved nodes (i.e., detected by SLN biopsy)	No
pN2b	Two or three tumor-involved nodes at least one of which was clinically detected	No
pN2c	One clinically occult or clinically detected tumor-involved node	Yes
pN3	Four or more tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with two or more tumor-involved nodes, or any number or matted nodes without or with in-transit, satellite, and/or microsatellite metastases	
pN3a	Four or more clinically occult tumor-involved nodes (i.e., detected by SLN biopsy)	No
pN3b	Four or more tumor-involved nodes, at least one of which was clinically detected, or presence of any number of matted nodes	No
pN3c	Two or more clinically occult or clinically detected and/or presence of any number of matted nodes	Yes

Note: pN1b, 2b, and 3b subcategories are dependent on clinical information that may be unavailable to the pathologist. If this information is not available, the parent category (pN1, pN2, or pN3) should be selected.

Lymph Node Sampling:

For histologic examination, whether for sentinel node analysis or for routine regional lymph node evaluation, *the entire node* should be submitted.

- Count lymph nodes identified (sentinel and non-sentinel).
- Specify site of lymph nodes identified.
- Measure lymph nodes in three dimensions or provide a range in size of greatest dimension if multiple.

- **For *macroscopically positive* lymph nodes**:

    - Describe the cut surface of the identified lymph nodes and the macroscopic appearance of tumor metastasis including the size and location within the node.
    - State if the metastatic focus is subcapsular and / or intramedullary.
    - State if matted lymph nodes are identified.
    - Give a precise size of the lymph node and size and location of the tumor implant.
    - Representative sampling of these lymph nodes is adequate.
    - Sample areas where extranodal extension is seen or cannot be excluded.
    - 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.

- **For small lymph nodes or *macroscopically negative* larger lymph nodes**:

    - Submit small lymph nodes (<5 mm) in toto.
    - Bisect larger lymph nodes or serially section larger lymph nodes at 2 mm intervals and entirely submit.
    - Submit the entire capsule of the lymph node for possible extranodal extension.
    - If the lymph node is large but macroscopically negative, submit sequentially so that a size calculation can be established.
    - 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:**

    - H&E-stained slides from multiple levels through serially sectioned sentinel lymph nodes increases the sensitivity of detecting microscopic melanoma metastasis.
    - Routine analysis (H&E-stained sections of the cut surfaces of a simply bisected lymph node) may lead to a false-negative rate of 10% to 15%.

METASTASIS ("M" of TNM)

- **Metastasis:**

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

pM Category	**pM Criteria**	
	Anatomic Site	**LDH level**
M0	No evidence of distant metastasis	Not applicable
pM1	Evidence of distant metastasis	See below
pM1a	Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph nodes	Not recorded or unspecified
M1a(0)		Not elevated
M1a(1)		Elevated
pM1b	Distant metastasis to lung with or without M1a sites of disease	Not recorded or unspecified
M1b(0)		Not elevated
M1b(1)		Elevated
pM1c	Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease	Not recorded or unspecified
M1c(0)		Not elevated
M1c(1)		Elevated
pM1d	Distant metastasis to CNS with or without M1a, M1b, or M1c sites of disease	
M1d(0)		Normal
M1d(1)		Elevated

Suffixes for M category: (0) LDH not elevated, (1) LDH elevated. No suffix is used if LDH is not recorded or is unspecified.

- Melanoma can metastasize to any organ site.
  - The regional lymph nodes are the most common site of metastases.
  - Distant metastases most commonly occur in the skin or soft tissues, the lung, liver, brain, bone, or gastrointestinal tract.

- In patients with distant metastases, the sites of metastases and elevated serum levels of lactate dehydrogenase (LDH) are used to delineate the M categories into three groups: M1a, M1b, and M1c, with 1-year survival rates ranging from 40 to 60%.

- Intralymphatic local and regional metastases may also become clinically manifest as:
  - Satellite metastases, defined as macroscopically visible cutaneous and / or subcutaneous metastases occurring within 2 cm of the primary melanoma, but not contiguous with the primary melanoma.
  - Microsatellites – microscopic and discontinuous cutaneous and / or subcutaneous metastases (> 0.05 mm in diameter) found on pathologic examination adjacent to a primary melanoma.
  - In-transit metastases, defined as clinically evident cutaneous and / or subcutaneous metastases identified at a distance greater than 2 cm from the

primary melanoma in the region between the primary and the first echelon of regional lymph nodes (not beyond the regional lymph nodes).
- These manifestations of melanoma increase the risk of both locoregional and distant metastases and portend a poor prognosis.

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