

Skin biopsy

Biopsy issues in specific diseases

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Learning objectives

After completing this learning activity, the learner should be able to identify the appropriate site for biopsy for direct immunofluorescence; identify the appropriate site and technique for biopsy for alopecia and vasculitis; and identify the appropriate technique for biopsy for pigmented skin lesions.

Disclosures**Editors**

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Misdiagnosis may result from biopsy site selection, technique, or choice of transport media. Important potential sources of error include false-negative direct immunofluorescence results based on poor site selection, uninformative biopsy specimens based on both site selection and technique, and spurious interpretations of pigmented lesions and nonmelanoma skin cancer based on biopsy technique. Part I of this 2-part continuing medical education article addresses common pitfalls involving site selection and biopsy technique in the diagnosis of bullous diseases, vasculitis, panniculitis, connective tissue diseases, drug eruptions, graft-versus-host disease, staphylococcal scalded skin syndrome, hair disorders, and neoplastic disorders. Understanding these potential pitfalls can result in improved diagnostic yield and patient outcomes. (*J Am Acad Dermatol* 2016;74:1-16.)

Key words: basal cell carcinoma; bullous diseases; connective tissue diseases and porphyria; cutaneous T-cell lymphoma; dermatofibrosarcoma protuberans; hair disorders; malignant melanoma; neoplasms; panniculitis; primary cutaneous B-cell lymphoma; staphylococcal scalded skin syndrome; squamous cell carcinoma; Stevens—Johnson syndrome; toxic epidermal necrolysis; vasculitis.

INTRODUCTION

Obtaining a skin biopsy specimen is one of the most common and important procedures performed by dermatologists, and histologic examination of a biopsy specimen may represent the most informative and cost-effective test in all of medicine—yet little curriculum time is devoted to teaching this important

procedure. Many textbooks describe the surgical aspects of skin biopsy techniques, so that will not be the focus of this article. Rather, we will address important practice gaps that can affect patient outcomes, with a focus on potential pitfalls involved in performing skin biopsy examinations when specific disease entities are suspected. Clinical entities for

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which site selection and biopsy technique can have a profound influence on results include bullous diseases, vasculitis, panniculitis, systemic diseases, such as lupus erythematosus (LE), dermatomyositis (DM), drug reactions, Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), staphylococcal scalded skin syndrome (SSSS), hair disorders, and neoplastic disorders (Table I).

Important practice gaps include inappropriate site selection leading to false-negative direct immunofluorescence (DIF), decreased diagnostic yield of alopecia and vasculitis biopsy specimens, and inappropriate technique leading to limitations in the interpretation of pigmented lesions and patterns of nonmelanoma skin cancer.

BULLOUS DISEASES

Key points

- **The sensitivity of direct immunofluorescence is superior to that of indirect immunofluorescence or enzyme-linked immunosorbent assay for the diagnosis of pemphigoid**
- **Nonbullous lesional or perilesional skin from the trunk is preferred for the diagnosis of pemphigoid**
- **Brief immersion in formalin produces false-negative results in pemphigus but not in most other bullous diseases**
- **Saline is superior to liquid nitrogen, Michel medium, and Zeus medium for direct immunofluorescence specimens delivered to the laboratory within 48 hours**
- **Extracted anagen hairs may be adequate to demonstrate diagnostic direct immunofluorescence findings in patients with pemphigus**

Accurate diagnosis of autoimmune bullous disease depends upon clinicopathologic correlation and supportive studies showing circulating autoantibodies and their pattern of deposition in skin or mucosa. In the setting of bullous pemphigoid (BP), DIF has been shown to be more sensitive than indirect immunofluorescence (IIF) or enzyme-linked immunosorbent assay (ELISA).¹ Of the commonly used assays, DIF is the most sensitive (90.8%), followed by IIF (76%) and ELISA (ranging from 59% for BP230 to 73% for BP180). ELISA assays for BP180 can be falsely negative in 7.8% of BP patients, because the antigen maps to regions outside of the NC16A domain.² While the specificities of all 3 assays are close to 100%, false-positive DIF can be associated with bullous scabies, representing an important pitfall, especially in older individuals in nursing homes.

In addition, the choice of biopsy site can have an effect on the yield of the biopsy specimen. Biopsy specimens for DIF of immunobullous disease should be taken from nonbullous lesional skin or uninvolved perilesional skin within about 1 cm of a bulla (Fig 1).^{3,4} Both bullous skin and uninvolved skin farther from the bullae are associated with a higher rate of false-negative results. Lower extremity skin should be avoided when possible because of a greater risk of false-negative results.^{5,6} The specimen should be taken from above the waist if possible, and some experts advise trunk skin over extremity skin. It should be noted that most data regarding biopsy site choice are from patients with BP.

Specimens for light microscopy should show an intact vesicle or bulla if possible. If small vesicles are present, removal of an entire lesion is preferred. For larger lesions, the specimen should be obtained from the edge of a blister and should contain both portions of the blister and intact skin so that the edge of the blister and inflammatory infiltrate can be seen. A punch biopsy specimen works well for small vesicles and for perilesional skin because it allows for evaluation of the full thickness of the epidermis and dermis. A scooped shave biopsy specimen that extends into the reticular dermis may be useful to harvest larger bullae intact. Specimens for light microscopy should be placed in formalin for preservation. Tissue obtained for DIF should not be placed in formalin; rather, Michel or Zeus media can be used for preservation.^{7,8} Some immunodermatology laboratories accept specimens transported on normal saline-soaked gauze, and saline solution preservation has been associated with higher diagnostic yields. Standards for specimen acceptance vary, and the dermatologist should review the standards of the laboratory being used (Table II). Some require that fresh specimens be received during laboratory working hours—preferably within 6 hours of obtaining them—or specimens flash-frozen with liquid nitrogen and transported on dry ice. While some laboratories warn that specimens should not be immersed in saline solution, some data suggest that saline is actually a superior transport medium for DIF specimens. In a recent study of 25 specimens comparing 3 transport media, a diagnosis was reached in 92% after 24-hour saline exposure, 83% after 48 hours in saline, 68% after freezing in liquid nitrogen, and 62% after 48 hours in Michel medium.⁹ Specimens transported in saline feature decreased background fluorescence and enhanced specific fluorescence. The saline-split IIF technique can be performed on specimens transported in saline or Michel or Zeus media.

Table 1. Suspected disease entities with recommended biopsy type, size, and requested laboratory tests

Disease	Recommended biopsy technique	Comments
Autoimmune bullous diseases	H&E—Saucerized removal of intact bulla if possible, or broad saucerization of periphery of bulla DIF—Perilesional skin ≤ 1 cm from bulla	Avoid lower extremity when possible because of delayed healing and greater risk of false-negative results
Epidermolysis bullosa	Saucerized removal of intact bulla if possible, or broad saucerization of periphery of bulla	Blisters >12 hrs old should be avoided; a fresh blister can be induced in clinically uninvolved skin, near a site where the patient usually blisters. Topical anesthetics should be avoided because they may induce artificial blistering
Vasculitis	H&E—Punch or deep shave of well-established purpuric lesion (>72 hrs old) DIF—Punch or deep shave of acute lesion (<24 hrs old)	IgA vasculitis is more likely to retain positive DIF findings in established lesions
Panniculitis	Deep incisional biopsy	Punch biopsy specimens tend to fracture, leaving inflamed or necrotic fat behind. An electric rotary power punch can overcome this limitation. A 6-mm punch is the smallest size that should be divided for culture and H&E. The edge of a necrotic focus provides a high yield for culture and special stains. The skin surface should be prepped with alcohol and allowed to evaporate. Deliver the culture specimen to the desk that handles fungal and AFB specimens
Lupus and dermatomyositis	H&E—Punch biopsy of an established lesion (>6 months old) that is still active DIF—Punch biopsy of lesional skin; choose an established lesion (>6 months old) that is still active	
SJS/TEN vs SSSS	Shave or punch biopsy including the full thickness of the epidermis	Desquamating sheets of skin may constitute an adequate specimen
Scarring alopecia	H&E— ≥ 4 -mm punch biopsy of an established lesion (>6 months old) that is still active DIF— ≥ 4 -mm punch biopsy of lesional skin; choose an established lesion (>6 months old) that is still active	For all forms of alopecia, avoid the active advancing border. Established lesions are preferred. One specimen can be bisected transversely 1 mm above the dermal/SQ junction, or it can be submitted intact for the laboratory to section transversely or with the HoVert or Tyler techniques. One specimen can be bisected vertically—half submitted in Michel medium for DIF and half added to the formalin bottle containing the transversely bisected or intact specimen
Nonscarring alopecia	For pattern alopecia or telogen effluvium— ≥ 4 -mm punch biopsy of an established area of alopecia For alopecia areata or syphilis— ≥ 4 -mm punch biopsy of an active lesion of recent onset is preferred.	If pattern alopecia or telogen effluvium is suspected, the specimen can be bisected transversely 1 mm above the dermal/SQ junction, or it can be submitted intact for the laboratory to section transversely or with the HoVert or Tyler techniques. For other forms of nonscarring alopecia, the specimen should be submitted intact
BCC/SCC	Shave or punch biopsy of adequate depth to show the invasive pattern and detect perineural invasion if present	In convex sites or thin facial skin, more superficial shave biopsy specimens may be appropriate. The skin should be pulled taught to provide greater control over depth. Avoid creating contour defects in sebaceous skin
Suspected melanoma DFSP	Complete excisional removal whenever possible Deep incisional biopsy	This may take the form of a saucerization

Continued

Table I. Cont'd

Disease	Recommended biopsy technique	Comments
CTCL	Broad shave biopsy specimens below the depth of the DEJ are superior to punch biopsies	
Primary cutaneous B-cell lymphoma	Deep incisional biopsy whenever possible	A punch biopsy specimen or saucerization does not allow assessment of architecture

AFB, Acid-fast bacilli; *BCC*, basal cell carcinoma; *CTCL*, cutaneous T-cell lymphoma; *DEJ*, dermoepidermal junction; *DFSP*, dermatofibrosarcoma protuberans; *DIF*, direct immunofluorescence; *H&E*, hematoxylin–eosin; *IgA*, immunoglobulin A; *SCC*, squamous cell carcinoma; *SJS*, Stevens-Johnson syndrome; *SQ*, subcutaneous; *SSSS*, staphylococcal scalded skin syndrome; *TEN*, toxic epidermal necrolysis.

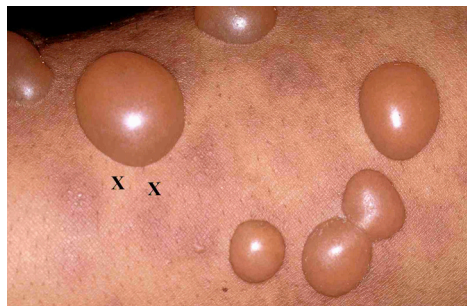


Fig 1. Biopsy specimens for direct immunofluorescence for suspected bullous pemphigoid should be taken from nonbullous lesional skin or uninvolved perilesional skin within 1 cm of a bulla.

In cases where the clinician or assistant has inadvertently placed a specimen for DIF into formalin, the specimen should immediately be removed and rinsed in normal saline. Evidence suggests that brief formalin immersion produces false-negative results for pemphigus, but not for diseases characterized by deposits at the dermoepidermal junction.¹⁰ Immunohistochemistry (IHC) for immunoreactants can be performed on formalin-fixed, paraffin-embedded tissue, but these immunostains are not widely available, and individual laboratories must validate the assays to determine the sensitivity and specificity relative to DIF. In the authors' experience, sensitivity decreases significantly when IHC methodology is substituted for DIF, and the assay should only be performed when a separate specimen for DIF cannot be obtained.

The root sheath of plucked anagen hairs may demonstrate positive immunofluorescence, especially in the setting of BP, and may prove adequate for diagnosis.¹¹ In this technique, an anagen hair is forcibly extracted from the scalp. The presence of a gelatinous follicular sheath above the hair bulb denotes an adequate specimen. For DIF biopsy specimens of mucosal surfaces, the tissue immediately adjacent to an erosion can be friable, and a tissue specimen from normal-appearing

mucosa 3 to 5 mm away may be preferable. For IIF studies, monkey esophagus is superior to human skin as a substrate, but the use of both substrates provides the maximum yield.¹²

For the diagnosis of inherited forms of epidermolysis bullosa (EB), immunofluorescent or immunohistochemical mapping can be used to localize the level of the split. In cases where mapping fails to demonstrate the level of cleavage, the diagnosis can be confirmed with transmission electron microscopy or mutational analysis. For electron microscopy, the specimen should be placed in a 2.5% glutaraldehyde solution (glutaraldehyde buffered by 0.1 M sodium cacodylate, pH 7.4) and stored at 4°C before overnight shipping at ambient temperature or with a cold pack if ambient temperatures are >37°C.

In most cases, mapping works quite well using antibodies to collagen IV and keratin 14. Specific monoclonal antibodies targeting EB-specific proteins are available in specialized laboratories. Information is available on the Dystrophic EB Research Association (DeBRA) International website (www.debra-international.org). The choice of biopsy location is key, because blisters >12 hours old may feature epidermal necrosis, proteolytic antigen degradation, or reepithelialization, resulting in a false assessment of the cleavage plane. A fresh blister can be induced in an area of skin that is clinically uninvolved, near a site where the patient usually blisters. The palms and soles should be avoided when possible because the increased skin thickness in these areas makes the induction of a blister and identification of the cleavage site more difficult. Topical anesthetics (eg, lidocaine 2.5% and prilocaine 2.5% under occlusion) should be avoided because they may induce artificial blistering, especially in the epidermis. Injectable anesthetics are preferred. Various methods have been used to produce the blister, including a cotton swab, pencil eraser, or gloved finger. Firm downward pressure is applied to the skin and traction is then exerted by twisting 180° in each direction until erythema is

Table II. Recommended handling and transport media

Test	Recommended transport media	Comments
H&E	Formalin	The specimen should ideally be fixed in ≥ 10 times the specimen's volume worth of formalin (overnight or first 24 hrs), then it can be placed in a smaller amount of formalin for shipping. Do not fill whirl pack bags more than half full for shipping. When shipping during the winter months, isopropyl alcohol should be added to the formalin in a 1:10 ratio to prevent freezing artefact
DIF	Normal saline, liquid nitrogen, Michel medium, or Zeus medium	Normal saline provides the highest yield if the laboratory accepts it, and it can be delivered within 24-48 hrs without freezing. Specimens transported in liquid nitrogen must not be allowed to thaw
Microorganism culture	Nonbacteriostatic saline	Deliver specimens promptly so they can be processed or refrigerated. Make arrangements with the laboratory and avoid shipping over weekends. If fungi are to be isolated, the tissue should be diced rather than ground. The fungal/AFB bench typically processes tissue in this fashion, while routine cultures are commonly ground with glass beads
Electron microscopy	2.5% glutaraldehyde solution	Store at 4°C before overnight shipping at ambient temperature or with a cold pack if ambient temperatures are $>37^{\circ}\text{C}$
Flow cytometry	Fresh specimen submitted on saline-soaked gauze or RPMI medium	

AFB, Acid-fast bacilli; DIF, direct immunofluorescence; H&E, hematoxylin–eosin; RPMI, Roswell Park Memorial Institute.

produced. For patients with mild skin fragility, ≤ 2 minutes of friction can be necessary to induce the blister. The biopsy specimen is obtained after a lag time of at least 5 minutes after the induction of erythema to allow the development of a microscopically identifiable blister. The biopsy specimen should include the border of erythematous and nonerythematous skin so that the split is clearly shown. As an alternative, children can be asked to perform an activity that typically induces a fresh blister just before the clinic appointment for obtaining a biopsy specimen. In patients with extreme fragility, the twisting motion of the punch biopsy itself may be sufficient to induce the cleavage plane necessary for diagnosis.

VASCULITIS

Key points

- Biopsy specimens should show both the post-capillary venule and the deep plexus, especially for septic, rheumatoid, and antineutrophil cytoplasmic antibody–associated vasculitides, which are more prone to involve deeper vessels
- For the highest yield, biopsy specimens for hematoxylin–eosin staining should be taken from an established purpuric lesion (ie, >72 hrs old)

- For direct immunofluorescence biopsy specimens, an acute lesion (<24 hrs old) provides the highest yield
- Immunoglobulin A vasculitis often retains positive direct immunofluorescence findings in established lesions
- The biopsy yield for temporal arteritis increases with Doppler localization and harvesting of a 2-cm segment

Biopsy specimens are helpful to distinguish vasculitis from nonvasculitic disorders and to distinguish between the different types of cutaneous vasculitis. In the setting of immunoglobulin A (IgA)-induced vasculitis, DIF studies are particularly helpful. Lesions <24 hours old are more likely to show immune reactants, and IgA is more likely to remain in vessels of established lesions compared to other immunoglobulins—although some data suggest that IgM may persist for up to 7 days in a significant number of specimens.¹³ The presence of IgA deposits correlates with a diagnosis of Henoch–Schönlein purpura with gastrointestinal, renal, and joint manifestations.¹⁴ Fibrin leakage is found in any vascular injury and is a prevalent but nonspecific finding. For light microscopy, biopsy specimens obtained from fully evolved lesions are more likely to have all of the diagnostic features of leukocytoclastic vasculitis. Biopsy specimens of

evolving lesions that are <24 hours old are likely to have some infiltration of neutrophils with karyorrhexis, but often do not show expansion of the vessel wall or fibrin deposition. Septic vasculitis frequently features fibrin thrombi, endothelial necrosis, and the involvement of deeper arterioles. The latter 2 features are shared with rheumatoid and antineutrophil cytoplasmic antibody–associated vasculitis. Other forms of vasculitis are more likely to affect only the postcapillary venule with retention of an intact endothelial layer.¹⁵ After 48 hours, the inflammatory infiltrate in leukocytoclastic vasculitis begins to shift from a neutrophilic infiltrate to include lymphocytes and macrophages, but karyorrhectic debris and fibrin remain for extended periods.¹⁶

A well-developed purpuric lesion (ie, between 24 hrs and 1 week old) is usually adequate for hematoxylin–eosin-stained specimens, and we prefer a lesion that has been present for about 72 hours. The biopsy specimen should be obtained from the center of the lesion. In patients with livedo racemosa, a biopsy specimen should be obtained from the pale center of an erythematous ring. This is where the occluded vessel is most likely to be seen. When ulceration is present, the biopsy specimen should be taken from the trailing edge of the ulcer, rather than the ulcer itself, because any ulcer bed will demonstrate nonspecific vasculitis. If vasculitis involving a large muscular vessel is suspected, a deep incisional biopsy with step sections may be appropriate. Temporal artery biopsies should ideally be at least 2 cm, because specimens <0.7 cm may compromise the diagnosis.¹⁷ Doppler localization of the artery can be of help in planning the biopsy procedure.

PANNICULITIS

Key points

- A deep incisional biopsy provides the highest yield
- An electric rotary power punch or using the double punch technique are alternatives, especially in patients with a bleeding diathesis where a smaller biopsy specimen may be of benefit
- Gelfoam hemostasis is helpful for patients with a bleeding diathesis
- A 6-mm punch is the smallest size that should be divided for culture and hematoxylin–eosin staining
- The culture specimen should be diced rather than ground when attempting to isolate fungal and acid-fast bacillus organisms

Dermatologists are skilled surgeons and are well-suited to perform the deep incisional biopsies

typically needed for a definitive diagnosis of panniculitis.¹⁸ The surgery is generally well-tolerated, but potential risks must be taken into account, including scarring, infection, and poor wound healing. In situations where the history and physical examination lead to a high probability of a single diagnosis, the biopsy may not be in the patient's best interest. For example, classic erythema nodosum in a child with no other signs or symptoms is most likely related to previous streptococcal infection, and obtaining a biopsy specimen is not likely to change management. Lipodermatosclerosis is another diagnosis that can often be established with a fair degree of certainty without obtaining a biopsy specimen. When a biopsy is needed in this setting, the effect of stasis on delayed wound healing should be discussed with the patient as part of routine counseling before the procedure. In contrast, the histologic confirmation of erythema induratum, pancreatic panniculitis, infectious panniculitides, or subcutaneous panniculitis–like T-cell lymphoma can be critical to proper patient management. A deep incisional biopsy is typically needed, because punch biopsy specimens commonly fracture at the level of the inflamed fat, leaving the diagnostic portion of the specimen at the base of the wound. In select patients, including those with bleeding diatheses, a “power punch” (Fig 2) can overcome this obstacle and produce a diagnostic biopsy specimen with a small wound. Hemostasis can then be obtained with the use of Gelfoam (Pfizer, New York, NY) and gentle pressure. Electronic power punches were once commonly used to obtain hair transplant grafts, and many dermatology departments still have the equipment sitting in a storage closet. If the engine has worn out, the long metal punch can be attached to a variable speed Dremel tool placed in a plastic bag to comply with standard blood precautions. The rapid circular torque of the power punch typically produces a long intact cylinder of fat, even in patients with lobular necrosis. The major drawback of this technique is that the specimen is just 4 mm in diameter, and the pathologist may not be able to evaluate the full architecture and inflammatory pattern of the panniculus. In situations where it is difficult to obtain an intact specimen of necrotic fat, tissue culture and touch preparations for histologic examination may still lead to the correct diagnosis.¹⁹

CONNECTIVE TISSUE DISEASES AND PORPHYRIA

Key points

- Punch biopsy specimens should be ≥ 4 mm in diameter

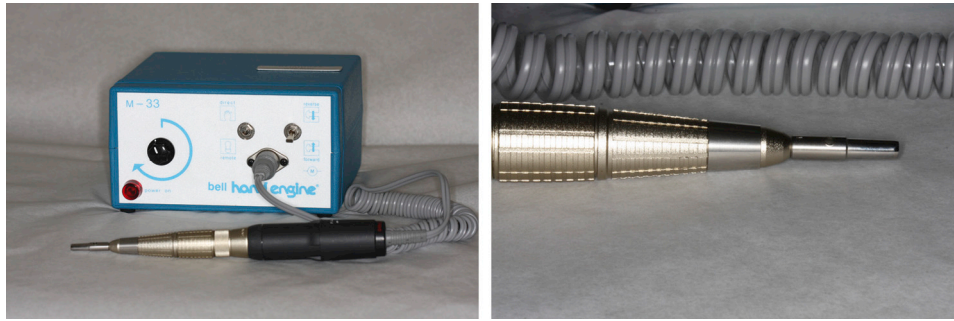


Fig 2. An electronic power punch is a good alternative for biopsy specimens obtained from patients with panniculitis, especially in patients with a bleeding diathesis. (Photograph courtesy Howard Pride, MD, Geisinger Medical Center.)

- **In contrast to immunobullous disease, direct immunofluorescence of perilesional skin is of no value in most patients with connective tissue disease, because immune deposits are only present in lesional skin. One exception is the lupus band present in normal sun-protected skin in patients with active systemic lupus who are at risk of renal disease. This test has largely been replaced by assays for double-stranded DNA antibodies that identify the same population**
- **In the setting of chronic cutaneous lupus erythematosus, a punch biopsy specimen of an established lesion (>6 months old), but still active, provides the highest yield for both hematoxylin–eosin-stained sections and direct immunofluorescence**
- **Porphyria is commonly associated with hyalinization of superficial blood vessels which reveal strong immunofluorescence for immunoglobulin M and complement component 3**

A diagnosis of chronic cutaneous lupus may require identification of compact hyperkeratosis, follicular plugging, interface dermatitis, basement membrane zone thickening, dermal mucin, columnar lymphoid infiltrate involving fibrous tracts, nodular lymphoid infiltrates involving the superficial and deep vascular plexus, infiltrates in the eccrine coil, or subcutaneous nodular lymphoplasmacytic aggregates with fibrinous lobular fat necrosis. In short, the key diagnostic changes may be present anywhere from the stratum corneum to the deep subcutaneous fat. An optimal diagnostic specimen to show these features should be ≥ 4 mm in diameter and can often be obtained using the punch biopsy technique extending to the subcutaneous fat; however, in the case of lupus panniculitis, a deep incisional biopsy may be required. Dermatomyositis often produces more superficial atrophic skin



Fig 3. Biopsy specimens for direct immunofluorescence for suspected chronic cutaneous lupus erythematosus should be taken from an established lesion (>6 months old).

lesions, and a shave biopsy may be adequate to show the diagnostic findings.

In contrast to immunobullous disease, DIF of perilesional skin is typically of little value in patients with connective tissue disease. Immune deposits are present in lesional skin in the setting of chronic cutaneous LE, and are best shown in an established lesion >6 months old (Fig 3). In the setting of systemic LE, the lupus band test on normal sun-protected skin has largely been replaced by assays for double-stranded DNA antibodies that identify the same population at risk for renal disease.

While some data suggest that positive immunofluorescence in sun-exposed skin is detected in only about one-third of patients with subacute cutaneous LE (SCLE), other groups have found a higher incidence (86% of patients). In contrast, while fluorescent dust-like particles are characteristic, some data suggest they are found in a small minority of patients with SCLE.²⁰ Positive DIF is noted in the majority of patients with established lesions of discoid or hypertrophic LE, and in lichenoid lesions of hypertrophic LE, DIF is the

single best test to differentiate the disorder from hypertrophic lichen planus.

The lilac inflammatory border of morphea reveals characteristic nodular lymphoplasmacytic aggregates involving both the superficial and deep vascular plexus. A deep punch or incisional biopsy specimen extending to the level of the subcutaneous fat is required to show these findings. More advanced lesions of morphea reveal a punch biopsy specimen with parallel sides resulting from dermal hyalinizing fibrosis with a loss of space between collagen bundles and the loss of periadnexal fat. This contrasts with the tapered appearance of most punch biopsy specimens. As with inflammatory morphea, a deep punch or incisional biopsy specimen is optimal to show these features. A diagnosis of superficial or atrophic morphea can sometimes be established with a more superficial punch or saucerized (scooped) shave biopsy specimen, especially if the loss of CD34 dendritic cells can be seen in the dermis.^{21,22} Even in superficial morphea, the biopsy specimen should include at least the upper half of the reticular dermis.

A diagnosis of porphyria cutanea tarda is suggested clinically by the presence of scarring, milia, and hypertrichosis in sun-exposed areas. Biopsy specimens of an intact bulla obtained using the saucerized shave technique typically have the diagnostic features of caterpillar bodies (ie, trapped remnants of the basement membrane zone sandwiched between layers of epidermis), a subepidermal split, festooning of dermal papillae, hyalinized superficial dermal vessels, and solar elastosis. Similar skin changes may be seen in variegate and coproporphyrria. Erythropoietic protoporphyria features more extensive hyalinization of vessels without solar elastosis. In all forms of porphyria with vessel hyalination, DIF reveals strong vascular fluorescence with IgM, complement component 3, and often fibrin. Punch or scooped shave biopsy specimens that include at least the upper third of the dermis are adequate to show these findings. Transport media are chosen as for immunobullous disease.

STEVENS—JOHNSON SYNDROME, TOXIC EPIDERMAL NECROLYSIS, AND STAPHYLOCOCCAL SCALDED SKIN SYNDROME

Key points

- **The biopsy specimen must include the full thickness of the epidermis**
- **If the differential diagnosis includes fixed drug eruption, the biopsy specimen must**

also include both the superficial and deep vascular plexus

- **The roof of any old blister will become necrotic and mimic the full-thickness necrosis of erythema multiforme/toxic epidermal necrolysis; therefore, acute lesions are always preferred**

Stevens—Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are associated with high morbidity and mortality, and an accurate diagnosis requires clinicopathologic correlation. The characteristic histologic features include satellite necrosis of keratinocytes that frequently progresses to full-thickness necrosis. Both conditions have an acute onset and progress rapidly, and the stratum corneum retains its normal basket weave architecture. A sparse perivascular lymphoid infiltrate is characteristically present, but features of fixed drug eruption (FDE), such as papillary dermal fibrosis, a polymorphous superficial and deep infiltrate with eosinophils, and perivascular melanophages are lacking. In patients with a clinical presentation characteristic for SJS or TEN, the roof of an acute blister or sloughed skin may be adequate for diagnosis, but when the differential diagnosis includes generalized FDE, the biopsy specimen must extend to the level of the subcutaneous fat so that both the superficial and deep vascular plexus can be assessed. Old bullae of any cause feature epidermal necrosis and can mimic SJS/TEN, so an acute lesion is preferable for biopsy whenever possible.

Staphylococcal scalded skin syndrome (SSSS) produces a split at the level of the stratum granulosum via acantholysis, which can be seen in biopsy specimens that extend to the level of the mid-epidermis. A sample of sloughed skin may be adequate to show the diagnostic features in many patients. The condition typically affects children because of their limited ability to eliminate the toxin. Adults with renal failure can also develop the disease, and in this population the major histologic differential diagnosis is pemphigus foliaceus. The 2 conditions can be identical in routine histologic sections but can be differentiated by DIF, IIF, or by a positive ELISA. It is likely that all media suitable for transportation of pemphigoid specimens would also be suitable for transport of pemphigus specimens. A major difference is that even brief immersion in formalin will completely extinguish pemphigus immunofluorescence, so a specimen inadvertently dipped into a formalin bottle must be discarded and a new specimen must be obtained.

When SSSS is suspected, appropriate cultures should be obtained from the suspected focus of infection. Intact bullae are sterile, but denuded areas of skin are prone to secondary bacterial colonization. Lesions of pemphigus foliaceus also become secondarily colonized within 1 to 2 days, so tissue culture of chronic skin lesions are of little value in the differential diagnosis.

HAIR DISORDERS

Key points

- **More than 1 biopsy specimen may be needed to establish the diagnosis**
- **For all forms of alopecia, the active advancing border should be avoided because established lesions provide a higher diagnostic yield**
- **The punch should be ≥ 4 mm in diameter**
- **Place the punch at the same angle as the emerging hairs to avoid transecting hair follicles**
- **Hair density in different ethnic groups is now well established, so biopsy specimens of normal scalp for comparison are of limited value**
- **Transverse sections offer a higher diagnostic yield for pattern alopecia and telogen effluvium**
- **Serial vertical sections offer a higher diagnostic yield for most other forms of alopecia, including scarring alopecia**
- **Combining vertical and transverse sections provides the highest yield**
- **For lupus direct immunofluorescence specimens, choose an established but still active lesion (>6 months old)**

To maximize the diagnostic yield of a scalp biopsy, a 4-mm punch biopsy specimen should be obtained from a well-developed lesion, preferably of several months' duration but still active (Fig 4). For DIF specimens in suspected LE, the lesion should be ≥ 6 months' duration. When biopsy specimens of active lesions are nondiagnostic, a biopsy specimen from a scarred area can be helpful diagnostically when evaluated with elastic tissue stains or polarized microscopy.²³⁻²⁶ Chronic cutaneous LE produces broad areas of scarring, whereas lichen planopilaris and folliculitis decalvans produce focal wedge-shaped scars at the level of the follicular infundibulum. Central elliptical alopecia of black women (the major form of central centrifugal cicatricial alopecia) is characterized by broad fibrous tract remnants with retention of the surrounding elastic sheath, contraction of the dermis, and thick

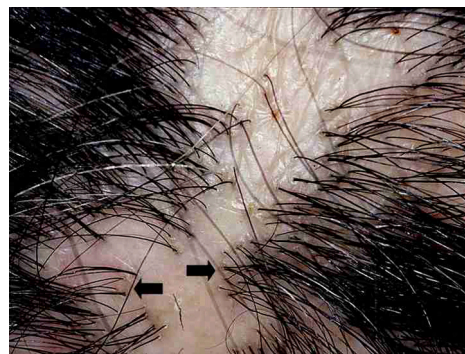


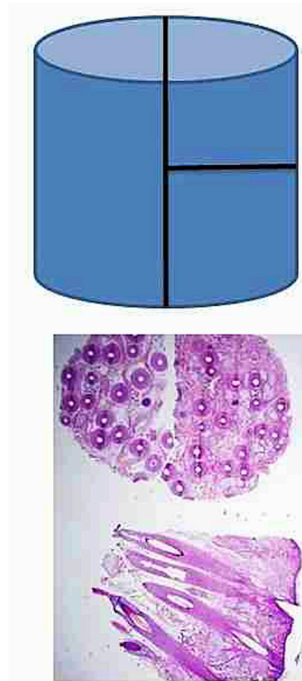
Fig 4. Biopsy specimens obtained from patients with scarring alopecia should be taken from well-developed lesions, preferably of several months' duration, but that are still active. Fully developed lesions provide a higher diagnostic yield when compared with the advancing border.

recoiled elastic fibers. Fluorescent microscopy of H&E-stained sections shows the pattern of elastic tissue without the need for a special stain, provided that excessive eosin is not used during the staining process. With polarized microscopy, fibrous tracts do not demonstrate birefringence in contrast to the birefringence of normal dermal collagen. A pattern of collagen birefringence sparing the fibrous tract remnants demonstrates good specificity for nonscarring alopecia, but the sensitivity is relatively low.²⁷

Both trichoscopy and confocal microscopy can be useful to increase the diagnostic yield through better site selection, directing the clinician to established areas of active inflammation.²⁸⁻³⁰ The punch should enter the scalp at the same angle as the emerging hairs to avoid transecting follicles. Hemostasis is easily obtained with Gelfoam, and the appearance of a punch biopsy scar that heals by secondary intention after Gelfoam hemostasis is generally superior to that after suturing of the biopsy site.

In the setting of scarring alopecia, diagnostic yield can be improved if 2 biopsy specimens are taken from developed lesions.^{31,32} One is bisected vertically, with one half placed in appropriate immunofluorescence media and the other half in formalin. The second specimen may be added to the same bottle intact or bisected transversely, 1 mm above the dermal/subcutaneous junction. A label should be placed on the bottle alerting the laboratory if the specimens are already bisected and that all 3 pieces should be embedded in a single cassette, cut side down. This technique provides the advantages of vertical and transverse sections at no added cost, because only one 88305 pathology Current Procedural Terminology code is reported for the 3 pieces of tissue in a single bottle.

Tyler Technique



HoVert Technique

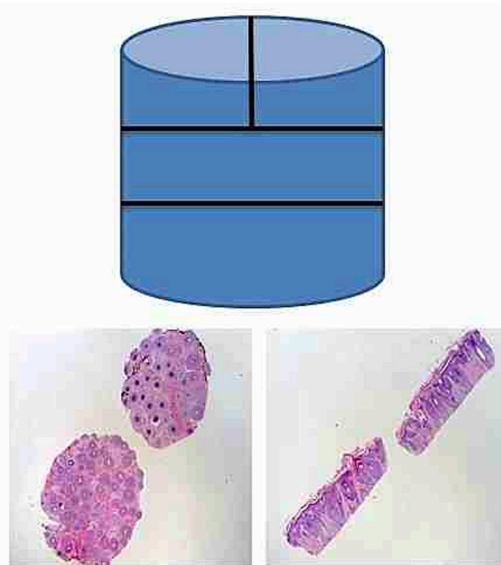


Fig 5. The Tyler technique involves vertical sectioning of the specimen, followed by transverse sectioning of one half. The 2 resulting half circles are embedded facing one another, fresh cut side down. The HoVert technique involves transverse sectioning of the specimen followed by vertical sectioning of the upper 1-mm portion.

Often, the laboratory only receives a single specimen. When the differential diagnosis is focused on pattern alopecia or telogen effluvium, transverse sections are superior and allow for accurate assessment of the proportion of anagen, catagen, and telogen follicles and the relative proportion of terminal and vellus hairs.^{33,34} For most other forms of alopecia, including all forms of scarring alopecia, vertical sections provide a higher yield than transverse sections alone.³⁵⁻³⁸ Serial sectioning of the specimen can increase the diagnostic yield.³⁹ Various techniques have been used to obtain the benefits of both vertical and transverse sections from a single specimen. The HoVert technique involves transverse sectioning of the specimen followed by vertical sectioning of the upper 1-mm portion. This allows better visualization of the follicular infundibulum—the site of involvement in lichen planopilaris.⁴⁰ The Tyler technique involves vertical sectioning of the specimen, followed by transverse sectioning of one half. The 2 resulting half circles are embedded facing one another, fresh cut side down. The technique results in the appearance of 1 vertical section next to 1 transverse section. In serial cuts, half of the transverse specimen demonstrates levels towards the subcutaneous fat, while the other half

demonstrates serial levels towards the epidermal surface (Fig 5).⁴¹ It should be noted that in conditions such as diffuse chronic telogen effluvium, >1 biopsy specimen at different points in time may be needed to establish the correct diagnosis.⁴²

NEOPLASMS

Key points

- Shave biopsy specimens often fail to show an underlying invasive pattern or perineural invasion
- A broad shave biopsy frequently produces surrounding erythema and can create both an indistinct tumor border and the appearance of a growth that is larger than the original cancer
- Curettage specimens rarely allow for adequate assessment of tumor growth characteristics
- Punch biopsy specimens are more predictive of growth pattern but may not be suitable for all body sites
- If a shave biopsy specimen is obtained, the presence of spiky irregular tumor islands, fibroblast-rich stroma, or sclerotic red stroma suggest a more aggressive underlying growth pattern

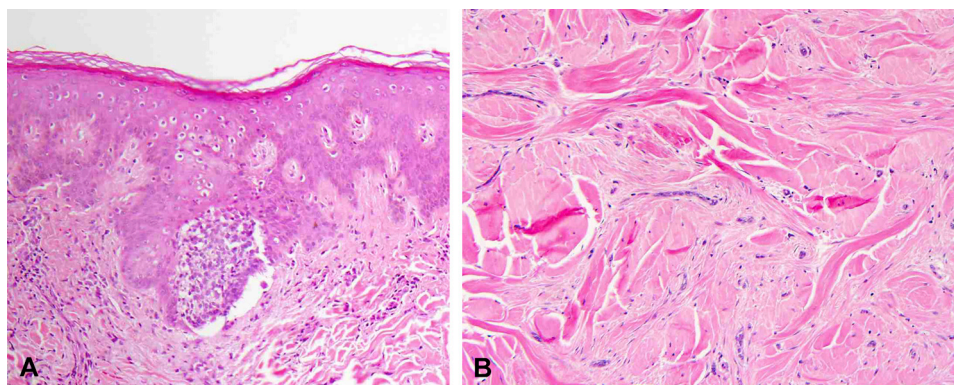


Fig 6. Superficial biopsy specimens of basal cell carcinoma may feature a superficial multifocal growth pattern (A) and may fail to show a deeper, more aggressive growth pattern (B).

Basal cell carcinoma/squamous cell carcinoma

A biopsy specimen is typically obtained before definitive treatment of suspected nonmelanoma skin cancer to confirm the diagnosis and because treatment decisions may be altered based on the growth pattern of the tumor. Visual examination, curettage, and dermoscopy have been used to define tumor extent before definitive surgery, but their accuracy is limited,⁴³ suggesting that scouting biopsies may be of use for poorly defined tumors that are not suitable for Mohs micrographic excision. Superficial and nodular basal cell carcinomas (BCCs) are best suited to defining tumor extent via curettage, whereas infiltrative, morpheiform, and micronodular BCCs are surrounded by dense collagen, and the extent of the tumor cannot be defined by curettage of tumor and stroma.^{44,45} The same is true for desmoplastic squamous carcinoma and for tumors with perineural invasion, which may extend far beyond the clinically evident tumor margins.

The shave technique is commonly used in the setting of suspected BCC or squamous cell carcinoma, but a small (2-3 mm) punch biopsy specimen obtained from the center of the lesion may have advantages over a shave biopsy specimen.^{46,47} First, the tumor margins remain visible and distinct after performing a punch biopsy, whereas a broad shave biopsy frequently produces surrounding erythema that can create an indistinct tumor border or the appearance of a lesion that is larger than the original cancer. This could result in a larger than necessary excision to remove both the tumor and surrounding erythema. Punch biopsy specimens may also be superior to define the growth pattern of the tumor. Specifically, micronodular, morpheiform, or infiltrative features may be present only in the deeper portions of a tumor (Fig 6).^{48,49} If observation and palpation suggest a superficial or nodular BCC or Bowen disease, shave biopsy may be

the preferred technique. The shave technique can be performed more quickly, costs less in supplies compared to alternative biopsy techniques, requires less instrumentation, does not require suture closure, and the resulting bleeding can be stopped with chemical cautery. In contrast, when a more aggressive growth pattern is suspected, a small centrally placed punch biopsy specimen can help to ensure the smallest definitive surgical excision of the tumor and the most appropriate therapeutic approach based on histologic subtype.

Either technique is superior to biopsy via curettage, which rarely allows accurate assessment of tumor growth characteristics. Regardless of the biopsy technique used, if spiky irregular tumor islands, fibroblast-rich stroma, or sclerotic red stroma are present, they suggest a more aggressive underlying growth pattern, and a deeper biopsy may be prudent before beginning definitive therapy.⁵⁰

Malignant melanoma

Key points

- **Complete excisional removal is the method of choice for suspected melanoma whenever possible**
- **For macular lesions on the trunk, saucerization can achieve complete excisional biopsy and is often preferred by patients**
- **When the differential diagnosis is melanoma vs dysplastic nevus, a saucerization with a 0.5- to 2-mm margin of normal skin allows for assessment of the lesion and ensures a high likelihood of removal with clear margins**
- **The specimen can be scored, inked, or tagged at 12 o'clock to allow for orientation of the specimen**
- **Partial biopsy specimens are associated with a lower diagnostic yield, but there is no**

evidence that tumor colonization of a deep punch biopsy wound worsens prognosis

- **In the setting of lentigo maligna, a broad thin shave biopsy or multiple small shave biopsies offer a higher diagnostic yield than a punch biopsy**
- **An incisional biopsy is an excellent alternative when it can be oriented along a naturally occurring skin crease**
- **For multicolor lesions, each color in the lesion should be sampled**
- **Acral lesions should be bisected perpendicular to the dermatoglyphs (finger print lines) to avoid the artifactual appearance of confluence**

Pigmented lesion biopsy may be the single most important intervention performed by dermatologists, because early detection is a key factor in determining prognosis in patients with melanoma. Complete excision is the method of choice for suspected melanoma when feasible because it allows the pathologist to judge symmetry and overall architecture.⁵¹ Partial biopsy specimens can lead to sampling error and an erroneous diagnosis, but may be performed in large lesions where complete excisional biopsy is impractical. The tumor may colonize deep punch biopsy wounds, but there is no evidence that such colonization is associated with a poorer prognosis. The greatest limitation of partial biopsy specimens is that they may compromise both diagnostic accuracy and staging.⁵² Evaluation of the remaining neoplasm after subsequent excision leads to tumor upstaging in roughly 21% of patients, with 10% subsequently qualifying as candidates for sentinel lymph node biopsy.⁵³ In a study of 157 cases of biopsy-proven melanoma in situ, subsequent excision revealed invasive disease in 8.3% of the lesions.⁵⁴

When feasible, excisional biopsy specimens should be oriented along the longitudinal axis on the extremities, because this reflects the pattern of lymphatic drainage and spread. Another option to minimize total tissue removed at the time of biopsy is to remove the lesion following the outline of the lesion itself with 1- to 3-mm margins. This can be performed in the manner of a Mohs micrographic surgery layer or via razor blade saucerization. For macular or clinically shallow lesions, the plane of saucerization is within the dermis, and primary closure is unnecessary. The sample provides the pathologist with the entire lesion, and the resulting shallow wound and round scar is often preferred by patients. The specimen can be scored, inked, or tagged at a designated site, such as 12 o'clock, to

allow for orientation of the specimen during gross examination and the subsequent histologic assessment of the lesion.⁵⁵ Full-thickness wounds may be closed primarily with temporary sutures without removing any additional tissue (ie, dog ears), because optimal cosmetic closure can be addressed after the definitive excisional surgery with margins.^{56,57} When closing the surgical defect of a suspected melanoma, undermining of the wound edges should be minimized, because this could theoretically affect sentinel lymph node mapping.

Lentigo maligna deserves special mention; the large size of the lesion often precludes complete excision. Misdiagnosis is common in small specimens because of the lack of effacement of rete ridges, areas of regression, and collision with nonmelanocytic pigmented lesions, such as benign lentiginosities and pigmented actinic keratosis.⁵⁸⁻⁶⁰ Punch biopsy specimens are associated with a high rate of false-negative results.⁶¹⁻⁶³ In the authors' experience, selection of the darkest portion of the lesion identifies the area with greatest pigment incontinence but is not necessarily the most diagnostic portion of the lesion. A broad thin shave biopsy specimen resembling properly cut prosciutto can provide the pathologist with a broad view of the junctional melanocytic proliferation without creating a deep wound. An excellent alternative may be multiple small shave biopsy specimens that sample every color and morphology within the lesion. These can all be placed in a single specimen bottle to maximize the chance of correct diagnosis and minimize cost, because only a single 88305 Current Procedural Terminology code will be billed. An elliptical incisional biopsy specimen is an excellent alternative when it can be oriented along a naturally occurring skin crease to hide the resulting scar.

Nevi on volar skin are often shallow and may be completely removed via saucerization. They are often characterized by elongated nests that follow the dermatoglyph furrows.⁶⁴⁻⁶⁷ Once removed, they should be bisected perpendicular to the dermatoglyphs to avoid the false appearance of junctional confluence. If you cannot trust your laboratory to do this, you should do it yourself and indicate that the specimen is already bisected.

Dermatofibrosarcoma protuberans

Key points

- **A deep incisional biopsy specimen is required to show the growth pattern of dermatofibrosarcoma protuberans in subcutaneous tissue**

- **Immunostains can be helpful in the diagnosis of indeterminate superficial biopsy specimens**

The pattern of infiltration into the subcutaneous tissue is a key diagnostic feature of dermatofibrosarcoma protuberans, and a deep incisional biopsy specimen is required to show this pattern. More superficial biopsy specimens can resemble cellular dermatofibroma, and immunostains or a second biopsy specimen may be required to establish a definitive diagnosis.^{68,69}

Cutaneous T-cell lymphoma

Key points

- **Broad shave biopsy specimens that include a wide area of the dermoepidermal junction are preferred to show the architecture of the infiltrate**
- **The epidermotropic population is the target for molecular studies, and broad biopsy specimens are also superior for immunostaining profiles and gene rearrangement studies**
- **Biopsy specimens from different anatomic sites that show an identical clone are helpful to establish the diagnosis in indeterminate cases**

Key diagnostic features of mycosis fungoides, the most common form of cutaneous T-cell lymphoma, include broad zones of papillary dermal fibrosis, vacuolar interface dermatitis with a lymphocyte in nearly every vacuole, and epidermal lymphocytes that are large, angulated, and hyperchromatic when compared to benign recruited dermal lymphocytes in the superficial dermis. A superficial perivascular infiltrate that spares the underside of the postcapillary venule is also characteristic. For the pathologist to see these diagnostic features, the biopsy specimen should extend to below the postcapillary venule (ie, to the upper reticular dermis). A gently saucerized broad shave specimen can be ideal, although punch specimens are often adequate. Because the malignant infiltrate is epidermotropic, more DNA for molecular studies can be extracted from broad shave specimens than from punch specimens. Shave specimens also provide a broader field of involvement for comparison of lymphoid populations in immunohistochemical studies. Plastic embedding of the biopsy specimen is sometimes employed, because it allows for 1- μ m thick sections that can show the cerebriform nuclear structure.⁷⁰

Clonality of the T cell population can be established by means of polymerase chain reaction (PCR)

or Southern blot analysis of T-cell receptor gene rearrangements (TCRs). While fresh tissue has a slightly higher yield, these studies can now be performed on formalin-fixed paraffin-embedded tissue. While most cases of mycosis fungoides express the α/β receptor on the cell surface and stain with the BF-1 antibody, genomic rearrangements are commonly detected in the gamma chain genome because of the relative genetic simplicity of this portion of the genome. Testing for beta chain TCR can also be helpful, especially when the assay for the gamma chain demonstrates a germline configuration.

Any immune response is, by its nature, a clonal phenomenon, but the clones are quite small and the majority of T cells in a benign infiltrate are polyclonal T cells recruited by a small clone of memory T cells. In contrast, lymphomas feature expansion of a clonal population. Demonstration of an identical clone at multiple sites is highly suggestive of mycosis fungoides.⁷¹ When clonality in the skin matches that in the blood, mycosis fungoides is also highly likely. This is more likely to be seen in patients with advanced disease, but can also be detected in some patients with early disease.⁷²⁻⁷⁴

Immunophenotyping usually confirms T-cell origin (CD3⁺) with strong predominance of CD4 over CD8⁺ cells. Double negative or double positive CD4/CD8 phenotypes may occur, and the deletion of mature T cell antigens (such as >90% deletion of CD7 relative to CD3 in the epidermotropic compartment) is supportive of the diagnosis of MF.⁷⁵

Primary cutaneous B-cell lymphoma

Key points

- **Architecture of the infiltrate and zonal immunostaining patterns is critical to the diagnosis and is difficult to assess in small or superficial specimens**
- **A large incisional biopsy specimen is preferred**
- **If a smaller biopsy specimen is more appropriate for a given patient, a saucerization or deep punch specimen is superior to a standard shave biopsy specimen**

A skin biopsy providing an adequate specimen for histopathologic examination is key to the diagnosis of lymphoma. Incisional or punch biopsy specimens are generally preferred, but some sessile nodules can be removed effectively via saucerization, avoiding the crush artefact that sometimes accompanies punch biopsy specimens. If a punch is used, the biopsy should extend into the subcutaneous tissue, and a 6-mm punch is preferable when possible.

Small or superficial biopsy specimens impair adequate assessment of architecture and depth of involvement.⁷⁶

The specimen may be fixed in formalin because IHC, chromogenic in situ hybridization to establish kappa and lambda light chain restriction, and PCR-based gene rearrangement studies can be performed on formalin-fixed paraffin-embedded tissue.⁷⁷ If flow cytometry is to be performed, a fresh specimen submitted on saline-soaked gauze or Roswell Park Memorial Institute medium is preferred.

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