

Improving diagnostic accuracy of skin biopsies

Paul Stevenson, Karl Rodins

Background

The skin biopsy is a simple but essential clinical skill of the general practitioner. Performed properly, it can be quick and comfortable for the patient, and yield a very high level of diagnostic information. Performed incorrectly, it can lead to delays in diagnosis and treatment for the patient.

Objective

This article reviews some simple but effective steps the clinician can take to ensure proper technique and maximise the diagnostic accuracy of their skin biopsies.

Discussion

Diagnostic accuracy can be improved through optimal selection of biopsy site, correct biopsy technique, requesting ancillary tests where appropriate, proper handling of specimens, and providing detailed clinical information for the dermatopathologist.

THE SKIN BIOPSY is an essential tool for the management of skin conditions. Performed properly, it can help establish a diagnosis and guide appropriate treatment.

The treating clinician can dramatically improve the diagnostic accuracy of biopsy specimens by:

- choosing optimal biopsy site
- performing correct biopsy technique
- obtaining adequate tissue sample
- where indicated, taking additional biopsy samples for ancillary tests
- ensuring proper handling of biopsy specimen and using the correct transport medium
- providing detailed clinical information for the dermatopathologist on the request form.

The most common biopsy types are punch, shave, excisional and incisional. Each technique has its own advantages and yields different information.

Choosing a lesion and site to biopsy

The choice of biopsy site is particularly important in inflammatory conditions (rashes) where multiple lesions and/or skin sites may be available for biopsy. Generally, established lesions with the most primary inflammatory change should be chosen, as early lesions may reveal non-specific features.¹⁻³ An exception to this is blistering (bullous) disorders, pustular eruptions and suspected vasculitis, where early lesions biopsied within 48 hours of appearance show more specific features on histopathology.^{1,2,4,5}

Older lesions with secondary skin changes such as crusting, scarring, regeneration and infection should be avoided, as they will have a lower yield.¹⁻³ Avoid biopsying lesions that have been excoriated, traumatised or recently treated,

as the underlying pathological process will be obscured.³

For small, inflammatory lesions (<4 mm), a punch excision could be performed. For large inflammatory lesions, choose the edge of an expanding lesion, the area of most colour change, or the thickest portion of the lesion.¹⁻³ Annular plaques should be biopsied at the elevated edge of the lesion.⁶

With an ulcer or ulcerated lesion, include a portion of normal adjacent tissue, because tissue from the ulcer bed alone often yields nonspecific findings. Best practice is to perform an incisional biopsy of the junction of ulcer and adjacent normal tissue.^{4,7}

Multiple biopsies should be considered where the rash has a polymorphic appearance, selecting sites of varying morphology.^{4,6} Some dermatoses also require additional biopsies for ancillary tests (refer to discussion below).

Selecting a biopsy site is a balance between choosing representative tissue and avoiding problem-prone areas. Certain sites on the body should be avoided when other sites are available. Where possible, avoid cosmetically sensitive areas, such as the face and areas prone to hypertrophic scarring such as shoulders, chest and breasts.¹⁻³ Avoid biopsying the legs and foot as venous stasis changes can affect interpretation and healing is often poor, especially in the elderly, diabetics and patients with vascular insufficiency.² The axilla and groin are higher risk sites for infection and should be avoided.³ Biopsies of bony prominences or pressure-bearing areas can have interpretive challenges and are more likely to be traumatised during movement.⁶ Generally, the thighs, abdomen, back and arms are good areas to biopsy.^{2,6} Table 1 provides a summary of biopsy recommendations for common dermatological presentations.

Table 1. Recommended biopsy technique for specific clinical presentations^{1,3-5,8,12}

Clinical presentation	Recommendations
Macular rash	Punch biopsy of centre of established lesion
Papular rash	Punch biopsy of centre of established lesion
Annular rash	Punch biopsy of advancing edge/raised border of established lesion Consider obtaining scrapings for fungal culture
Petechial rash or vasculitis	Punch biopsy of early purpuric lesion for H&E; additional punch biopsy of early lesion for DIF
Vesicobullous disorder	Punch biopsy of a small early intact vesicle for H&E. Otherwise, sample the edge of a large lesion, trying to keep the roof intact. Additional punch biopsy of perilesional skin for DIF
Cutaneous lupus (or other connective tissue disorder)	Punch biopsy of central part of an active established lesion (>6 months) for H&E. Additional punch of central part of lesion for DIF
Deep pathology (eg ulcer, panniculitis)	Deep elliptical incisional biopsy. For an ulcer, perform at the junction of ulcer and adjacent normal tissue
Suspected melanoma	Excisional biopsy of entire lesion with a 2 mm margin. If clinical suspicion is low, a deep shave excision including a margin of normal skin is acceptable
Lentigo maligna	Shave excision of the entire lesion if possible. Multiple shaves to remove the entire lesion is acceptable. Partial biopsy is strongly discouraged given heterogeneous nature of melanocytic lesions
Superficial solitary lesions (BCC, SCC or seborrhoeic keratosis)	Shave biopsy of entire lesion if small, portion of lesion if large
Keratoacanthoma	Excisional biopsy, if feasible, as well differentiated SCC cannot be excluded without assessment of the entire lesion
Alopecia	For scarring alopecia, choose an area with reduced hairs rather than no hairs at all. An additional biopsy for DIF can also be taken to assess for discoid lupus For non-scarring alopecia, choose area of greatest hair loss. Take two 4 mm punch biopsies for H&E (for vertical and horizontal sections) in the direction of hair growth
Scabies	Punch biopsy proximal edge of burrow

BCC, basal cell carcinomas; DIF, direct immunofluorescence; H&E, haematoxylin and eosin; SCC, squamous cell carcinomas

Preparation of site and anaesthesia

The essential equipment required for biopsies is listed in Table 2. Begin by marking out the biopsy site with a surgical marker as it may become obscured after injection of local anaesthetic. For most punch or shave biopsies, 0.5 mL of local anaesthetic is sufficient. Too much anaesthetic can distort the histological appearances and simulate dermal oedema.⁷ For large excisional or incisional biopsies, infiltrate a few millimetres outside the marked excision site to anaesthetise where sutures will be thread. Lignocaine 1% is the most commonly used local anaesthetic agent. It also comes

mixed with adrenaline in a 1:100,000 concentration, which prolongs anaesthesia effect and reduces bleeding.⁸

Clean the skin with an antiseptic agent such as isopropyl alcohol, chlorhexidine or povidone-iodine solution. Be gentle when cleaning the skin surface so as not to disturb the overlying scale, as keratin layers can sometimes contain diagnostic information.⁷ Chlorhexadine should not be used around ears due to risk of ototoxicity or around eyes due to corneal irritation or ocular injury.⁹

Punch biopsy

Punch biopsy is the preferred method of sampling for most inflammatory

dermatoses because it allows the pathologist to examine all layers of skin from the epidermis through to the top layer of the subcuticular fat. For most punch biopsies, a 4 mm diameter punch provides adequate tissue sample for histopathology assessment.^{5,6,10} Smaller punch biopsies risk yielding insufficient tissue for accurate diagnosis and should be reserved for cosmetically sensitive areas.¹⁰ Consider a 5 mm punch in dermatoses with atypical features.⁶

For most inflammatory conditions, penetrating the punch instrument through reticular dermis into the subcutaneous fat is a sufficient depth. A feeling of 'giving way' marks the point of reaching the subcutaneous fat. Most punch instruments

Table 2. Equipment for biopsies^{1,3-5,8,12}

Equipment	Comments
Surgical marking pen	
Skin preparation solution	Isopropyl alcohol, chlorhexidine, or povidone-iodine
Gauze	
Sterile drape	
Gloves	Non-sterile is sufficient for shaves or punches
Local anaesthesia	1% lignocaine +/- 1:100,000 adrenaline. Sodium bicarbonate in a 1:9 ratio can be added to lignocaine to reduce pain
3 ml or 5 ml syringe	
Drawing up needle 18 or 21 gauge	
Injecting needle 25 to 30 gauge	Pain can be minimised with slow injection of anaesthetic and a small-gauge needle
Biopsy instrument:	
• Punch (4 mm or 5 mm)	For punch biopsy
• Flexible shave instrument or scalpel blade	For shave biopsy
• Scalpel holder + No. 15 scalpel blade	For excisional or incisional biopsy
Closure instruments (punch/excision/incision):	
• 3.0–5.0 non-absorbable sutures	Absorbable sutures may also be required for deep excisions/incisions in areas of high skin tension
• Forceps	
• Needle holder	
• Scissors	
Specimen containers in transport medium	10% formalin for routine haematoxylin and eosin microscopy Sterile urine jar with saline gauze or Michel's medium for direct immunofluorescence Sterile jar for microbiological culture
Haemostatic agents	Aluminium chloride or aluminium sulphate with cotton bud applicators Electrocauterisation device
White soft paraffin	
Adhesive dressings	

have a plastic hub that stops the punch going too deep.

Some sources recommend rotating the punch instrument in one direction as twisting back and forth may shear off fragile epidermis from dermis or increase the risk of 'double-cutting' the surface.¹ However, if gentle care is taken then twisting is permissible, in our view.

Once sufficient depth is reached, remove the punch instrument and apply downward pressure either side of the wound to elevate the core for its edges. Lift the biopsy core with a hypodermic needle or very gently with a pair of

forceps to prevent crush injury, which may affect interpretation. Free the specimen from the subcutaneous fat with iris scissors or scalpel blade.

In our view, punches 4 mm or more usually require closure with one or more interrupted sutures and will heal with a linear scar. Applying tension to the skin perpendicular to lines of least skin tension with your non-dominant hand during the procedure will leave an oval-shaped wound that is easier to suture closed. Punches 3 mm or less can usually heal with secondary intention without sutures.

Partial punch biopsies should not be performed on pigmented lesions, because the partial biopsy specimen may not represent areas of atypia or malignancy. Margin assessment is also more difficult to appreciate with punch biopsies because of how the pathology technician may dissect the specimen.⁸

Shave biopsy

Shave biopsy is the preferred method for solitary lesions that are raised or where pathology is confined to the epidermis.^{1-5,10} They are generally considered appropriate

for superficial basal cell carcinomas (BCC) and squamous cell carcinomas (SCC).^{11,12} The ideal shave biopsy depth requires practice, and a balance between diagnostic accuracy and cosmetic outcome. Too superficial will be inadequate tissue for diagnosis. Too deep will delay healing and increase scarring. A sample to the level of the superficial dermis is usually sufficient.^{2,7,12}

Shave biopsy is most easily performed using a flexible, gripped blade. Pathology labs often provide these consumables for free. Otherwise, a size 15 scalpel blade or scissors may be used. Make your first cut at 45 degrees angle to the skin at the edge of the lesion, moving the blade in a perpendicular motion.¹² Once sufficient depth is achieved, flatten the blade to 0 degrees and continue moving the blade from side to side until the lesion is removed.

Haemostasis can be achieved using pressure, aluminium chloride 20% in alcohol ('Driclor'), aluminum sulphate in aqueous solution ('Stingose'), or electrocauterisation.^{12,13} Caution is advised when using electrocauterisation subsequent to aluminum chloride in alcohol due to its flammable nature.

Wounds heal by secondary intention and no sutures are required. Aluminum chloride solution can be applied with a cotton-tipped applicator several times with concomitant pressure. This generally scars less than electrocauterisation and is less cumbersome.

Curettage is a form of shave biopsy resulting in a fragmented specimen that hampers histopathological assessment. If using curettage to treat a lesion, we recommend sampling the lesion with a standard shave biopsy for diagnosis prior to curettage.

Excisional and incisional biopsy

An excisional biopsy is an appropriate technique for suspected melanomas, subcutaneous or deep dermal tumours, and deep inflammatory processes.^{2,4,7,8} It requires more time and skill than other biopsy techniques, but yields more tissue for the dermatopathologist and allows for multiple studies if required.¹ The biopsy is performed like any standard excision by

removing an elliptical piece of skin with a scalpel blade and closing the wound with sutures. For a pigmented lesion, this should include the entire lesion with a 2 mm margin of normal skin.¹⁴ Ultimately, the excision must go to the level of subcutaneous fat for optimal histological diagnosis.^{2,4,7,8,15}

Incisional biopsy is appropriate for deep inflammatory processes (eg panniculitis), ulcers, porokeratosis, cutaneous lymphoma and medium-vessel vasculitis.^{2,4,7,8} It may also be appropriate where a large shave biopsy would leave an unacceptable scar on a cosmetically sensitive area, or where a punch biopsy does not provide sufficient representative tissue of the lesion.² The technique is similar to an excisional biopsy, except only a portion of the lesion is biopsied and the sample should include a 1 mm area of normal adjacent skin at one end of the ellipse.^{7,8} The ellipse does not need to be as wide as an excision, but it should extend deep into the subcutaneous tissue to sample fatty tissue.^{2,4,7,8,15}

The pathology request form and ancillary tests

Detailed completion of the pathology request form is one of the most overlooked but important aspects of the biopsy procedure. Detailed clinical information and an accurate macroscopic description of the skin lesion(s) will greatly aid the dermatopathologist with interpretation and assist in arriving at a diagnosis that correlates with the clinical picture. Without this, the pathologist may report differential diagnoses that do not match the clinical impression, leading to confusion and delaying correct management.⁵ Studies have shown that taking the time to fill out the pathology request form with sufficient clinical detail results in higher rates of correct diagnosis.¹⁶ Important clinical information to include on the request form is listed in Box 1.

If ancillary testing is required to assist with diagnosis, this should be stipulated on the request form, otherwise the technician may not prepare the specimen appropriately. The most commonly used ancillary tests in dermatopathology are

direct immunofluorescence (DIF) and microbiological culture.^{7,8} A separate, additional biopsy specimen should be taken for ancillary testing if it is indicated. Microbiological culture should be considered if an infective process is suspected.⁵ Indications for DIF include bullous disorders, lupus erythematosus (or other connective tissue disease) and cutaneous vasculitis.^{4,5} In cutaneous lupus, the biopsy for DIF should be taken from the representative lesions.⁴ For vesiculobullous disorders, biopsies for DIF should be from perilesional skin (normal skin within 1 cm of an active lesion) because the immune deposits can degrade in forming a vesicle, resulting in a false negative result.^{4,7}

The provision of digital images (both clinical and dermoscopic) to the dermatopathologist may aid in diagnosis, particularly in the diagnosis of atypical-appearing lesions or challenging melanocytic lesions. This could be considered routine practice in the digital age.

Handling and transport of specimens

Improper handling and transport of specimens can damage tissue samples and impair the accuracy of histopathology interpretation and diagnosis. Handle specimens carefully to minimise crush injury. Ensure the tissue specimen is

Box 1. Key information to include on the pathology request form for any biopsy

- Patient age and gender
- Precise anatomical site of the biopsy
- Morphological description of the lesion(s), including any evolution in appearance
- Distribution of lesion(s)
- Duration of lesion(s)
- Clinical impression and differential diagnosis
- Prior skin biopsy result(s) or dermatological diagnoses
- Medications if drug eruption is a differential diagnosis
- Other relevant patient clinical history

placed in the correct transport medium for the test you are requesting. Haematoxylin and eosin (H&E) light microscopy is the routine test for most skin biopsies and should usually be transported in 10% buffered formalin solution.

Tissue for DIF can be submitted in a sterile pot with saline-soaked gauze. Delays in specimen collection and processing can result in reduced diagnostic accuracy. Keep this in mind if performing a biopsy on a Friday afternoon and specimens will not reach the pathology lab until Monday. Tissue for DIF can also be submitted in a transport medium such as Michel's medium when there is less urgency in processing the specimen. Tissue for microbiological culture can be submitted fresh in a sterile container. If unsure, consult your pathology provider.

The sample pot(s) should be labelled with patient identification details, the precise anatomical body site of the biopsy, time and date of biopsy, and all information checked against the request form for consistency.

Postoperative care

Good postoperative care will reduce the risk of complications and improve the long-term cosmetic appearance. Risk factors for wound complications include immune-compromised patients, smoking, and biopsies in the axilla, groin or below the waist.¹⁰ Evidence supporting the routine use of oral antibiotics for biopsies is lacking.¹⁷ If the patient is immunosuppressed or the biopsy site is high risk for infection, a single dose of cephalosporin antibiotics (within three hours of procedure) may be considered.^{15,17} Biopsy sites should be dressed with a moist occlusive dressing and paraffin ointment that should remain undisturbed for 24 to 48 hours.^{10,15,17,18,19} Thereafter, until re-epithelialisation is complete, wounds should be gently cleaned daily with warm water and reapplication of paraffin ointment with nonstick dressing.^{10,15,18,19} Topical antibiotics are no more effective than white soft paraffin.^{18,19} Sutures, if present, should be removed in 5–7 days for the face, 12–14 days for back and legs, and 7–10 days for other areas generally.^{10,12,15}

Follow-up should be arranged to review wounds and discuss biopsy results.

Conclusion

The skin biopsy is a simple but essential clinical skill of the general practitioner. Performed properly, it can be quick and comfortable for the patient, and yield a very high level of diagnostic information. If unsure how best to perform the biopsy or if the lesion is in a high-risk area, specialist referral may be indicated. Where the histological findings are inconsistent with the clinical suspicion, request a second opinion from the same pathology provider. Where the clinical and histopathological findings combined do not formulate a clear diagnosis, specialist referral to a dermatologist is recommended.

Authors

Paul Stevenson MD, Medical Officer, Princess Alexandra Hospital, Brisbane, Qld; Associate Lecturer, School of Medicine, Griffith University, Gold Coast, Qld; Associate Lecturer, Faculty of Medicine, University of Queensland, Brisbane, Qld. paul.stevenson@griffithuni.edu.au

Karl Rodins FACD, FRCPA, Specialist Dermatologist, Queensland Institute of Dermatology, Brisbane, Qld
Competing interests: None

Provenance and peer review: Not commissioned, externally peer reviewed.

References

1. Alguire PC, Mathes BM. Skin biopsy techniques for the internist. *J Gen Intern Med* 1998;13(1):46–54.
2. Sleiman R, Kurban M, Abbas O. Maximizing diagnostic outcomes of skin biopsy specimens. *Int J Dermatol* 2013;52(1):72–78. doi: 10.1111/j.1365-4632.2012.05731.x.
3. Zuber TJ. Skin biopsy techniques: When and how to perform a punch biopsy. *Consultant* 2012;52(6):1–5.
4. Elston DM, Stratman EJ, Miller SJ. Skin biopsy: Biopsy issues in specific diseases. *J Am Acad Dermatol* 2016;74(1):1–16. doi: 10.1016/j.jaad.2015.06.033.
5. Sina B, Kao GF, Deng AC, Gaspari AA. Skin biopsy for inflammatory and common neoplastic skin diseases: Optimum time, best location and preferred techniques. A critical review. *J Cutan Pathol* 2009;36(5):505–10. doi: 10.1111/j.1600-0560.2008.01175.x.
6. Nischal U, Nischal Kc, Khopkar U. Techniques of skin biopsy and practical considerations. *J Cutan Aesthet Surg* 2008;1(2):107–11. doi: 10.4103/0974-2077.44174.
7. Whitehead K. Cutaneous biopsies. Taringa: Sullivan Nicolaidis Pathology, 2014. Available at www.snp.com.au/media/188842/cutaneous_biopsies.pdf [Accessed 1 August 2017].

8. Harvey NT, Chan J, Wood BA. Skin biopsy in the diagnosis of inflammatory skin disease. *Aust Fam Physician* 2017;46(5):283–88.
9. Lai P, Coulson C, Pothier DD, Rutka J. Chlorhexidine ototoxicity in ear surgery, part 1: Review of the literature. *J Otolaryngol Head Neck Surg* 2011;40(6):437–40.
10. Yang S, Kamp J. Common dermatologic procedures. *Med Clin North Am* 2015;99(6):1305–21. doi: 10.1016/j.mcna.2015.07.004.
11. Cancer Council Australia and Australian Cancer Network. Clinical practice guide: Basal cell carcinoma, squamous cell carcinoma (and related lesions) – A guide to clinical management in Australia. Sydney: Cancer Council Australia and Australian Cancer Network, 2008.
12. Pickett H. Shave and punch biopsy for skin lesions. *Am Fam Physician* 2011;84(9):995–1002.
13. Howe N, Cherpelis B. Obtaining rapid and effective hemostasis: Part I. Update and review of topical hemostatic agents. *J Am Acad Dermatol* 2013;69(5):659. doi: 10.1016/j.jaad.2013.07.014.
14. Cancer Council Australia, Australian Cancer Network, Ministry of Health New Zealand. Clinical practice guidelines for the management of melanoma in Australia and New Zealand. Wellington: Cancer Council Australia, Australian Cancer Network, Ministry of Health New Zealand, 2008.
15. Robinson J, Hanke CW, Sengelmann R, Siegel D (editors). Surgery of the skin: Procedural dermatology. Chapter 14: Skin biopsy techniques. Philadelphia: Elsevier Mosby, 2005; p. 203–12.
16. Comfere NI, Peters MS, Jenkins S, Lackore K, Yost K, Tilburt J. Dermatopathologists' concerns and challenges with clinical information in skin biopsy requisition form: A mixed-methods study. *J Cutan Pathol* 2015;42(5):333–45. doi: 10.1111/cup.12485.
17. Wright TI, Baddour LM, Barbari EF, et al. Antibiotic prophylaxis in dermatologic surgery: Advisory statement 2008. *J Am Acad Dermatol* 2008;59(3):464–73. doi: 10.1016/j.jaad.2008.04.031.
18. Del Rosso JQ. Wound care in the dermatology office: Where are we in 2011? *J Am Acad Dermatol* 2011;64(3 Suppl):S1–7. doi: 10.1016/j.jaad.2010.10.038.
19. Nijhawan RI, Smith LA, Mariwalla K. Mohs surgeons' use of topical emollients in postoperative wound care. *Dermatol Surg* 2013;39(8):1260–63. doi: 10.1111/dsu.

correspondence ajgp@racgp.org.au

