Skin Biopsies and Excisions

Skin biopsies are performed for diagnosis and also treatment, in some cases. This section presents the essentials and serves as a quick reference guide. For more detailed information on specific clinical situations and entities, please refer to the relevant documents. Depending on the clinical scenario, the options are: excisional biopsy (elliptical excision, saucerisation/shave excision, punch excision), incisional biopsy, superficial shave biopsy, punch biopsy and wedge biopsy. Curettage as a primary diagnostic procedure is not encouraged due to the limitations of the sample.

NON-PIGMENTED DISCRETE LESIONS

When malignancy is suspected and there are no high risk features*, **complete excision** with appropriate margins* is recommended.

Biopsy should be performed when the diagnosis is uncertain. Small and/or non-suspicious lesions can be removed by punch or shave biopsy if required.

*Refer to 1389 Squamous Cell Carcinoma, Guidelines for Management in the Primary Care Setting and 868 Basal Cell Carcinoma, Guidelines for Management in the Primary Care Setting.

Suspected keratoacanthomas should also be excised, if feasible, as the current recommended management is early excision. Distinction between keratoacanthoma and well-differentiated squamous cell carcinoma is often not possible on partial biopsy.

Saucerisation is ideal for broad lesions in sites that pose surgical or cosmetic difficulties, and in areas prone to keloid/hypertrophic scar formation.

Saucerised samples should be flattened on a piece of card to prevent excessive curling, which may impair assessment of margins.

Punch biopsy is preferable to superficial shave biopsy, particularly for thick keratotic lesions, as the latter is prone to sampling error.

Incisional or wedge biopsy may be used for large and deep lesions such as dermatofibrosarcoma protuberans, as assessment of the tumour interface with fat is essential for diagnosis.

For suspected lymphoma a separate biopsy should be sent for flow cytometry. This should be wrapped in saline soaked gauze or immersed in appropriate transport medium, such as RPMI, which is available from QML Pathology.

Such specimens should be marked "Urgent" and sent to the laboratory ASAP.

PIGMENTED LESIONS

Suspected melanoma should be excised with a 1-3mm margin as small or superficial biopsies may not be representative and may affect subsequent tumour staging.

Partial biopsies are appropriate:

- When the clinical suspicion of melanoma is low.
- In pigmented genital lesions as a significant proportion of these are benign melanotic macules that do not require disfiguring excision.
- For broad, suspicious lesions, or sites that pose surgical or cosmetic difficulties.

Consider sampling error and re-biopsy if a partial biopsy of a suspicious pigmented lesion yields a negative pathology report.

Saucerisation is suitable for complete removal of in situ/thin lesions. If any atypical/pigmented area is seen at the base following saucerisation, punch or elliptical excision of the area is required.

If partial biopsy of suspected melanoma cannot be avoided:

- Take one or more deep biopsies of the most suspicious, thickest areas
- For lentigo maligna-type lesions, multiple biopsies should be taken of each different area. In this instance, multiple small shaves are more appropriate than punch biopsies.

Dysplastic naevi that are not suspicious for melanoma do not need to be removed, but 'ugly ducklings' can be removed by saucerisation with a 0.5-1mm rim of normal skin beyond the pale brown halo.

Small suspicious acral lesions should be completely removed by saucerisation with a narrow rim of normal skin, as false positive results can arise from partial biopsy.

RASHES/INFLAMMATORY CONDITIONS

A **4mm punch biopsy** is most commonly used, followed by **incisional biopsy** for larger and deeper lesions. **Saucerisation** may also be used to remove entire blisters.

*If **infection** is suspected, a swab or separate sample should be sent fresh for microbiology.*

If the DIF sample is accidentally placed in formalin, remove immediately and rinse in saline.

The biopsy should be taken from lesions that have not been excoriated or ulcerated, and should include lesional as well as a small rim of perilesional skin. Frictional sites and lower limbs should be avoided due to potentially confounding secondary features and false negative results. Concurrent biopsy of normal skin may also be helpful in disorders of pigmentation.

More than one biopsy from different sites may be helpful, particularly if the rash has a polymorphous appearance.

In **blistering conditions**, the biopsy should be wide enough to **keep the blister roof attached**. A separate biopsy of a non-blistered lesion or normal skin immediately adjacent to the lesion should be taken and submitted in immunofluorescence transport medium for **direct immunofluorescence microscopy (DIF)**. If transport medium is not available, phosphate buffer saline is suitable.

A sample for DIF should also be considered for **lupus erythematosus**, **dermatomyositis** and **vasculitis**. For these conditions, biopsy site is crucial. For lupus and dermatomyositis, biopsy an established lesion (>6 months old) that is still active for both routine histology and DIF. For vasculitis, biopsy an established purpuric lesion (>72 hours old) for routine histology and an acute lesion (<24 hours old) for DIF.

If **panniculitis** is suspected, a deep or incisional biopsy is recommended to ensure that sufficient subcutaneous fat is included. Send a sample for culture if necrosis is seen at the time of biopsy.

For **annular lesions** (such as porokeratosis), incisional biopsies are ideal and should be taken from the centre of the lesion outwards to include a 1mm rim of normal skin.

When incisional biopsies of inflammatory conditions are taken, it is essential that both the biopsy type and reason for biopsy are stated clearly on the request form, as this affects the way the sample will be handled.

Clinical description of the rash, its distribution, duration, course and other history including medications is essential. Clinical photographs (sent via email or text message) are also helpful. A clinical differential diagnosis is also required for accurate categorisation, as many rashes show similar histological patterns.

ALOPECIA

Ideally, **two punch biopsies (4mm diameter)** should be taken **in a plane parallel** to the direction of hair growth/ emergence.

The **biopsies should be at least 5-6mm** in depth to include subcutaneous fat and the entire follicular unit (i.e. there should be no hairs emerging from the deep aspect).

Two biopsies are required to enable vertical and horizontal sections to be examined.

Where to biopsy?

- Non-cicatricial alopecia: Area of greatest hair loss.
- Cicatricial alopecia: An active affected area that is at least 6 months old (i.e. an area with reduced hairs rather than a completely bald area). Look for signs of inflammation (e.g. scaling or changes in pigmentation). Dermoscopy may be helpful for detecting this. A separate biopsy from the same area can also be sent for DIF.

Adequate **clinical information** is important including area of scalp involved, race, duration and clinical differential diagnosis.

Specimen Handling and Margin Assessment

The way that specimens are handled in the laboratory will depend on the type of sample, their size and shape, and whether they have been taken for inflammatory or neoplastic conditions. Marking sutures or nicks will also alter handling.

EXCISION SPECIMENS (e.g. elliptical, shave/saucerisation, punch)

These are inked on all surgical margins to enable histological recognition as such.

When specimen orientation has been specified, the specimen may be scored along a border or different ink colours may be used. A diagram is usually drawn and this can be helpful to clinicians when margins are involved.

The way the specimen is cut will depend on its size and shape.

- Most punch excisions and shave excisions are bi- or trisected, depending on their diameter. Even if such specimens have been orientated, margins can only be examined and measured in the plane of sectioning *(see figure 1)*. As shave specimens are generally cut along the longest plane to limit fragmentation, the margins assessed also tend to be the ones that are furthest from the lesion.
- Elliptical excisions that are approximately 10mm in maximum dimension can be cut in 3-4mm thick parallel 'bread loaf' slices and submitted completely *(see figure 2)*. For longer excisions, the entire lesion is submitted in parallel slices and longitudinal sections to the points of the ellipse are also submitted. This allows examination and accurate measurement of all margins *(see figure 2)*. For ill-defined lesions, the entire specimen is generally submitted in parallel slices.
- Other shapes are cut in a similar manner, with modifications *(see figure 3)*.
- Tiny ellipses (3-4mm) are generally treated as a small punch specimen and margins can only be accurately examined and measured in the plane of sectioning.
- Samples of pigmented lesions from acral sites are cut perpendicular to the dermatoglyphs (skin ridges/furrows) as parallel sections can result in a false positive result of melanoma as a result of profiling long, confluent junctional nests.

Figure 1 - Punch Excision

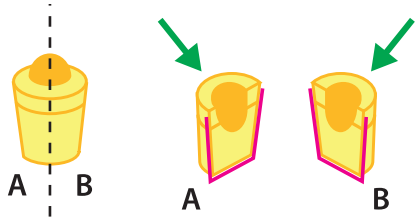
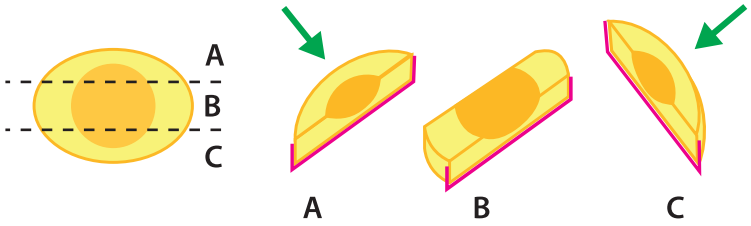


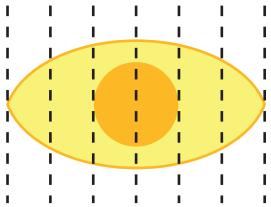
Figure 1 - Shave Excision



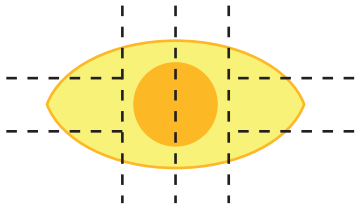
Margins assessable in histological section

Margins not assessable in the plane of sectioning

Figure 2

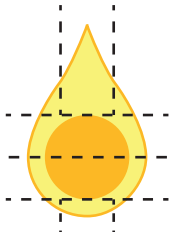


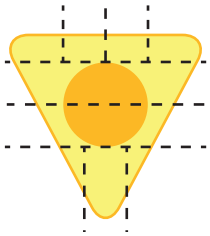
Parallel 'bread loaf' slices



Parallel slices of lesion with longitudinal slices to points of elipse

Figure 3





PARTIAL BIOPSIES (punch, shave, incisional)

- Small punch biopsies (up to 4mm) are submitted whole.
- Superficial shave biopsies are cut in parallel slices, usually in the longitudinal plane, to limit fragmentation.
- Incisional biopsies are mostly performed for inflammatory conditions and are either submitted in total without being cut, if no wider than 4mm, or they are bisected in the longitudinal plane to allow optimal visualisation of the inflammatory process.
- Requests for margins are sometimes received for such specimens and it should be noted that they are only reliable in the plane of sectioning.

MARGIN MEASUREMENTS

Histological margin measurements are provided to reflect the likelihood that a malignant tumour will recur. Although there is no absolute guarantee that recurrence will not occur, in general, this risk reduces with increasing distance of the margin. The Cancer Council Australia/Australian Cancer Network provides minimum histological margin recommendations for squamous and basal cell carcinoma. It should be noted, however, that there are limitations to the accuracy and reproducibility of histological measurements.

- **Tissue retraction:** This is more prominent in more elastic or muscular tissues, and less pronounced when solar damage is prominent. Tumour also retracts less than normal tissues.
- **Tissue sampling:** Accuracy increases with the number of tissue slices and histological sections examined. Each histological section is approximately 3-4µm thick, thus representing only 0.001% of each 3-4mm tissue slice. Even when multiple sections or levels have been examined, only a small percentage of the actual tumour has been examined.
- **Ill-defined margins:**
  - In some melanomas, single atypical melanocytes extend beyond the clinically visible lesion, making accurate or reproducible measurement virtually impossible.
  - There is often a continuum of changes in the spectrum of solar keratosis to in situ squamous cell carcinoma, which is often non-linear. Determining where such lesions start and end is poorly reproducible amongst pathologists.

Even when tumour is present at a margin, residual tumour may not be identified at re-excision. Positive margins also do not predict tumour recurrence. A variety of factors play a role, including tumour type, histological subtype and involved margin(s). Involvement of the deep margin confers almost double the risk of recurrence when compared to peripheral margin involvement.

EN FACE MARGIN ASSESSMENT

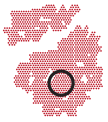
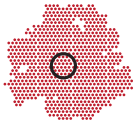
This is generally not performed on small, formalin-fixed specimens as both false positive and negative results can result from tissue retraction ex vivo. This method is most effective when used in Mohs micrographic surgery where the margins are assessed en face by frozen section. This allows a larger surface area to be assessed when compared to conventional parallel slices, resulting in lower local recurrence rates when narrow excision margins cannot be avoided.

Technical Details

Factors that may affect pathology results include the choice of biopsy site, technique and transport medium, as well as the lack of clinical details.

SELECTING THE BIOPSY SITE

Routine Histology

	Blotchy, macular	Annular	Discoid, plaque	Papular	Vesicular, bullous*	Nodule, tumour
Incision Biopsy						
Punch Biopsy		sometimes unsuitable for punch			unsuitable for punch	

*Whole blisters can also be removed by saucerisation.

For vesiculobullous rashes, vasculitis, lupus/dermatomyositis or scarring alopecia take a separate biopsy for **direct immunofluorescence microscopy** (IF):-

- Vesiculobullous rashes: Biopsy a non-blistered lesion or immediately adjacent (within 1cm) to a blister
- Vasculitis: Biopsy a fresh lesion <24 hours old
- Lupus/dermatomyositis: Biopsy an active established lesion (at least 6 months old)
- Scarring alopecia: Biopsy an active established lesion (at least 6 months old)

BIOPSY TECHNIQUE

1. Mark the site

- Allow an appropriate margin for punch/shave excisions

2. Skin preparation

- Be thorough but gentle, so that no scale or scab is rubbed off. Let alcohol dry before starting the biopsy.

3. Local anaesthesia

- Creating a wheal raises the lesion and makes it easier to shave.
- Lignocaine is most commonly used.
- Lignocaine with adrenaline helps control bleeding but should be avoided in certain sites.
- Recent evidence suggests that lignocaine with adrenaline can be safely used for digital anaesthesia unless the patient has peripheral vascular disease, connective tissue disease, Raynaud's disease or antiphospholipid syndrome.
- Over-infiltration with local anaesthetic can mimic dermal oedema.

4. Punch biopsies

- Choose the right punch size (excision vs partial biopsy; 4mm for inflammatory conditions)
- Determine the skin (Langer) line direction (see Fig 1) and stretch the skin perpendicular to the Langer lines to perform the biopsy. This will result in an oval that is easier to close.

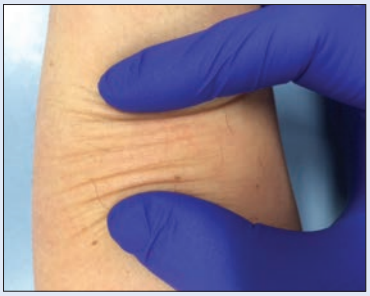


Fig. 1: Langer lines

- Place the punch tool over the lesion and gently but firmly rotate through the skin until there is a decrease in tension when it 'pops' into the subcutaneous fat.
- Remove the punch and gently lift the tissue with fine-toothed forceps or a skin hook/needle to minimise crush artefact.
- Cut the biopsy from the surrounding skin with scissors or scalpel blade.
- Close wound with a haemostatic agent† (if 4mm or less) or a suture or surgical adhesive.

†*Gelfoam, aluminium chloride 20% solution, Monsel solution, silver nitrate sticks*

5. Superficial shave biopsies or saucerisation

- For superficial shave, hold the blade parallel to the skin. Ensure that the biopsy is sufficiently deep for lesions with a thick crust.
- For saucerisation, hold the blade at a 45-degree angle to the skin and ensure that reticular dermis has been sampled.
- For pigmented lesions, if any pigment is seen at the base of the wound, remove this too.
- Dress with petrolatum. Keep moist and covered for at least a week to minimise scarring.

6. Incisional biopsies

- Make an elliptical incision about 2-3mm wide, cutting vertically down to fat.
- Grasp the biopsy by the deep edge using a skin hook or fine-toothed forceps.
- Cut the base of the biopsy with curved scissors or a scalpel
- Close the wound with sutures.

7. Wedge biopsies

- Make a deep V-shaped or triangular incision with a scalpel blade and remove the tissue with the tip of the blade.
- The biopsy should look like a triangular pyramid, with the base formed by the skin surface.
- The wound can be left to heal by secondary intention.

TRANSPORT MEDIUM

Routine histology: **Formalin**.

IF: **Immunofluorescence transport medium***

Saline can be used if Immunofluorescence transport medium is not available.
Remove and rinse the biopsy in saline immediately if it is accidentally placed into formalin.

Flow cytometry (for lymphoma): Use **RPMI medium*** or wrap the fresh specimen in saline-soaked gauze if not available.

*For further information or to order any of these media, please contact your local QML Pathology Laboratory.

CLINICAL NOTES

Don't forget to include details of the biopsy type, site and differential diagnosis. For inflammatory conditions, useful information includes duration, appearance, symptoms and medication history.

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