

RECOMMENDATIONS FOR OPTIMIZING QUALITY IN SKIN BIOPSY SAMPLES

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The skin biopsy is a power tool in the dermatologist's armament and is routinely used for diagnosis of skin diseases with unique light microscopic features. The clinician is challenged to take a representative sample from active lesions and to handle the sample in a way that will yield optimum results. The pathologist has to rely on the clinician's site selection to interpret those changes. The pathologist must also be careful to section and process the tissue so that as much information as possible can be obtained. It is important for the pathologists to understand how to take biopsies, even though their role comes in much later.

The veterinary clinician should have appropriate expectations from the biopsy procedure. Unlike tumor biopsies, the skin punch biopsy is far less likely to yield a simple diagnosis such as "mast cell tumor". Expect a morphologic diagnosis in at least 60-70% of your cases and use the biopsy information to (1) rule-out differential diagnosis (2) guide therapy (3) redirect the clinical work up and (4) establish a diagnosis in conjunction with the clinical lesions and history. Be aware that inflammatory skin biopsies are tedious and time-consuming and are charged at a slightly higher rate than tumor biopsies.

Recommendations on basic skin biopsy technique

The skin biopsy technique requires minor surgical and suture-tying skills. The challenge for the veterinarian is sample/tissue handling rather than patient care. Dogs and cats are the species most likely to be biopsied. Unlike humans, their thick pelage enables us to perform multiple biopsies with little risk of scarring. The following recommendations are considered standard by most dermatologists:

1. NEVER scrub the skin surface. In many cases, the diagnostic lesions are located in the corneal layer (e.g. pemphigus foliaceus). No one wants to admit that PF can be diagnosed from crusts alone, but it is true. The danger is that one could miss other dermal lesions (e.g. dermatophyte, demodex, lymphoma). I recommend that you put extra crusts into the formalin jar.
2. Submit four 6-mm punch biopsies per case (cats and dogs) unless the lesion is focal (e.g. vaccine reaction). 8-mm are great but may run a slightly higher risk of infection.
3. 4-mm punches should **only** be used for nasal planum, footpad specimens or very small lesions.
4. If you are using local anesthesia, make sure you put the lidocaine in the subcutis rather than the dermis. Injection in the dermis can cause artifact and the appearance of dermal edema.
5. Use a fresh punch for each animal. Dull punches cause tissue compression and artifact. Not all punches are created equal. We have found that some cheaper products are of poor quality (dull blade) and produce more artifacts.
6. Use the cutting action of the tool rather than pressure.
7. Handle the fresh specimen very GENTLY. Scoop the specimen up with forceps. Do not squeeze with forceps. Squeezing the cylinder with thumb forceps causes severe compression artifact (the appearance of a waist) and decreases diagnostic yield.

8. Blot and place immediately in 10% buffered formalin. Suture the pet after the sample is in formalin. Even 5 minutes under a surgical lamp can cause desiccation. Be careful when using empty blood tubes as a punch can get caught in the lid and undergo autolysis.
9. Do not place the sample in the cassettes used for biopsy processing and never place between sponges. These cause compression artifact if the sample is not completely fixed.
10. In winter months, allow samples to fix for 24 hours prior to shipping. This avoids freeze-thaw artifact if temperatures fall below 0 degrees C.

The line technique

When a punch biopsy arrives in the laboratory, the pathologist trims the sample with a razor or scalpel blade. The cylinder is gently bisected from the epidermis to the subcutis. Thin sections from edge of either half are what the pathologist ultimately examines microscopically. Keep in mind that two 5 µm sections of tissue are achieved from the 6-mm sample.

An ideal histologic section allows the pathologists to examine the hair follicle from the hair bulb to the os. If the sample is trimmed perpendicularly to the hair growth, then the hair follicles will not be in longitudinal section and all anatomic regions will not be visible (e.g. bulb, isthmus, infundibulum). In densely haired animals, the orientation for trimming is simple. When the animal is alopecic and/or lightly pigmented, it becomes very difficult to judge the direction of hair growth.

Dermatologists generally choose and mark the sites prior to sedation or local anesthesia. This technique is an easy method to mark sites and is particularly excellent for examining adnexal structures.

1. Use a fine or preferably ultrafine indelible marker (Sharpie® Sanford, Bellwood, IL, USA) to draw a 1-2 cm line in the direction of the flow of remaining hair. The marker does not rinse in formalin but is dissolved during the processing procedure.
2. Complete your procedure as you normally do. Write a note on the submission form that you used this technique.
3. The pathologist examines the formalin-fixed biopsy cylinder bisects the plug directly over your marked line.

Hints on Site Selection

The art of site selection develops with clinical experience. Even experienced dermatologists can be haunted by the failure to submit the most appropriate sample.

1. General practitioners and young veterinarians should attempt to biopsy every variation of lesions seen on the animal (3-4 sites are optimal), but submit more if needed. **Pustules, vesicles and crusts** have a high yield.
2. Do not sample marginal areas with a punch biopsy (especially bullae!). The lesion may be missed in trimming. Take a punch from the center of the lesion unless it is an ulcer. An elliptical biopsy is preferred for ulcers and bullae. You must include the adjacent unaffected skin.
3. Use excisional biopsies for subcutaneous/ pannicular lesions. It is not uncommon for the punch to miss the lesion entirely.

What is the influence of medical therapy?

If corticosteroids can cause eosinopenia and cause eosinophilic granulomas to disappear, then certainly these drugs are going to decrease eosinophils in tissue as well as alter or mask diagnostic lesions. If possible, withdraw all immunosuppressive and immunomodulatory drugs for at least two weeks prior to biopsy sampling. Injectable steroids will necessitate a longer withdrawal time depending on the drug used. Do biopsy without withdrawal if a life threatening condition exists.

Include a complete but concise clinical history

1. Historical Findings

A signalment and a complete but brief history are crucial to dermatopathology interpretations. Please do not photocopy the medical record with “see attached”. Most pathologists are not going to be able to interpret drug dosages but do benefit by knowing the prior therapy. Always include a lesion description and location on the animal. Also know that “lack of antibiotic response” is becoming less useful in this age of antibiotic resistance.

2. **Good quality images**- this is useful for the pathologist to compare with their interpretation.

3. **Histopathology Interpretation.** This may be given as either the “name of the disease” or a morphologic diagnosis. Although many skin disorders are readily diagnosed via histopathology, most require both clinical and histologic input to achieve a definitive diagnosis. Use the skin biopsy interpretation to help guide therapy and determine underlying disease processes.

4. **Lives of Lesions.** Unlike surgical biopsies (e.g. mast cell tumor), Please advise clients that the disease is a continuum; the changes seen at the microscope depend upon the stage at diagnosis. For example, two weeks after a thermal burn, the only change may be dermal fibrosis. Some cases will require repeat biopsy procedures as the diagnostic lesions may not be present at the initial consultation (e.g. pustule formation in pemphigus foliaceus).

5. Turn around time

Our turnaround time is typically 48 hours but may vary if we have a large teaching load or surgical pathology submissions. All packages should be sent via a mail carrier with tracking services.

6. **Second Opinion Slides** – Request unstained and ask for “charged” slides in case immunohistochemistry is needed.