ANIMAL HEALTH LABORATORY

*AHL LabNote* **Number 45** **February, 2016**

# Maximizing the benefit of surgical biopsies

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The key to a successful biopsy is to carefully identify, harvest and preserve the most diagnostically relevant lesions. A biopsy ideally provides a definitive diagnosis that guides case management. In situations where a definitive diagnosis is not obtained, histologic examination of lesions can be useful for ranking a list of differential diagnoses or may identify the likely pathogenesis (e.g., immune-mediated). A biopsy may be useful in several situations:

- To diagnose suspected neoplastic conditions.
- To confirm a diagnosis prior to selecting therapies which may be costly or potentially harmful (e.g., chemotherapy).
- To investigate unusual clinical presentations or persistent lesions (such as non-healing ulcers).
- To investigate conditions that appeared routine but have not responded to therapy.
- When differential diagnoses cannot be discriminated further by clinical examination or other less-invasive tests.
- When histopathology is required to diagnose the suspected disease (e.g., sebaceous adenitis).

**It is most rewarding to biopsy early in the course of a disease when the primary lesions are acute and plentiful**. This is especially true for skin conditions where chronic secondary lesions can mask the underlying problem. **It is recommended to biopsy before starting treatment and that anti-inflammatory treatments (glucocorticoids) are stopped 2-3 weeks prior to biopsy.** Secondary bacterial pyoderma complicates many dermatoses and should be treated prior to biopsy.

Biopsy submissions should include a pertinent, concise (and legible!) history with key physical exam findings, description of the lesions, differential diagnoses, and the results of relevant lab tests or therapeutic trials. Describe the type and distribution of primary and secondary skin lesions (Table 1) and mention specific locations that may be diagnostically important such as nasal planum, paw pads, and mucocutaneous junctions. **Clinical photographs are welcomed, and remember to include any specific questions that you want the pathologist to answer.**

Table 1. Primary and Secondary Skin Lesions

| | |
|---|---|
| **Primary lesions** | macule, papule, pustule, vesicle/bulla, wheal, nodule/tumor, cyst |
| **Secondary lesions** | epidermal collarette, excoriation, scarring, erosion/ulcer, fissures, lichenification, callus |
| **Lesions that may be primary or secondary** | alopecia, scale, crust, follicular casts, comedo, pigment changes |

It is important to **biopsy multiple sites** (e.g., 3-5 punch biopsies) that contain acute primary lesions as well as relevant secondary lesions. Secondary lesions that evolve from primary lesions may be diagnostic, whereas those that result from self-trauma are usually not. For example, in pemphigus

foliaceus, the pustules form crusts as they rupture and it is common to make this diagnosis based solely on the crusts!
In **depigmenting** skin conditions, the diagnostic lesions are often present at the site of active pigment loss (i.e., margin zone of grey skin). The cause of a cutaneous **ulcer** is often evident at the ulcer margin. In cases of **alopecia**, select samples from the center of the most alopecic regions as well as junctions with normal skin. (For suspected hair follicle diseases, mark the direction of the hair coat on the biopsy by drawing a line with a fine-point permanent marker – this allows the sample to be trimmed with optimal orientation of the hair follicles). With depigmenting and alopecic conditions, it can also be useful to include a region of normal skin for comparison. Remember to clearly identify different samples in separate jars (other ways to distinguish samples include sutures (good for tumors), tissue ink, and histology cassettes (for very small samples)).

**Punch biopsies** are effective for most skin conditions but an **elliptical excision** performed with a scalpel is preferred for fragile lesions, such as large pustules, diseases of the subcutaneous fat (panniculitis), tumors, and when attempting to sample the junction between abnormal and normal skin. Remember to not surgically prepare the biopsy site by scrubbing with antiseptics as this may remove diagnostic features such as crusts. **Ensure that the primary lesions are centered in the specimen** since punch biopsies are typically bisected down the center when sectioned at the laboratory (if you suspect that a lesion has been missed please contact the pathologist – it may be that additional sections are required).

Biopsies should be handled gently to minimize artifact and placed quickly into fixative (10% neutral buffered formalin, minimum **10:1 volume ratio** of formalin to tissue). Laser or cautery induces artifact and should be avoided for biopsies other than tumors. In the winter, **freezing artifact** during transport may be avoided by ensuring tissues are fully fixed (usually by 24 hours) and shipping in a solution of 9 parts formalin to 1 part alcohol. To minimize shrinkage or curling of larger skin biopsies, the tissue can be placed fat side down onto a wooden tongue depressor to stabilize the tissue during fixation.

For small tumors (< 1-2cm), submit the entire excisional biopsy for diagnosis and margin evaluation. Remember that formalin only penetrates approximately 1 cm into tissue - to promote fixation of larger specimens, it is helpful to partially incise the tissue at 1 cm intervals. For very large tumors (> 10 cm), representative samples of the mass and margins can be submitted for evaluation. **For complex samples**, such as amputated limbs, very large tumors, whole spleens, etc., please contact the laboratory in advance to discuss sampling and shipping with a pathologist.

**Evaluating completeness of excision is assisted by identifying relevant tumor margins with ink**. Surgical ink is commercially available in various colors for identifying the different margins of a resection. Margins may also be identified with sutures. Identifying margins is particularly helpful for large tumors that are often cut into smaller pieces for transport. To avoid ink leaching onto other tissues or margins, blot the margin with gauze to remove excess blood and liquid, apply the ink with a cotton swab, and allow the ink to dry for a few minutes prior to immersion in formalin.

**Key points to remember:** select multiple samples that contain primary (diagnostic) lesions, biopsy early, stop glucocorticoids 2-3 weeks prior to biopsy, treat secondary pyoderma first, handle and preserve tissues properly, include crusts, provide a concise relevant history, identify tumor margins, feel welcome to discuss your case with the pathologist.

Reference:
Bettenay SV, Hargis AM. 2006. *Practical veterinary dermatopathology for the small animal clinician*. Jackson, Wyo: Teton NewMedia.