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Dermatopathology Samples

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The New York State Animal Healthy Diagnostic Center at Cornell University, College of Veterinary Medicine is pleased to offer a specialized dermatopathology service. The goal of this service is to provide high quality interpretation of animal skin biopsy specimens from veterinary dermatologists, general veterinary clinicians, and university veterinary teaching hospitals by board certified veterinary pathologists and dermatologists with expertise in dermatopathology.

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Dr. Peters-Kennedy received her DVM from the University of Georgia in 2000. She then went on to complete a residency in anatomic pathology (2003), a small animal rotating internship (2004) and a residency in dermatology (2006) from Cornell University College of Veterinary Medicine. Dr. Peters-Kennedy is currently an Assistant Clinical Professor in the Department of Biomedical Sciences with an adjunct position in the Department of Clinical Sciences (Section of Dermatology) and in the Department of Population Medicine and Diagnostic Services. Dr. Peters-Kennedy has primary service responsibilities as a diagnostic dermatopathologist and clinical dermatologist, teaching responsibilities to residents and students and research responsibilities. Dr. Peters-Kennedy is an author and co-author of many peer-reviewed scientific articles and several book chapters and teaches dermatopathology to students as well as residents in anatomic pathology and dermatology.

Dermatopathology is a challenging area of pathology. Unlike most tumor biopsies, skin punch biopsy specimens of inflammatory skin disease are far less likely to yield a simple diagnosis. In most instances, the clinician should expect a morphologic diagnosis and a list of differential diagnoses. Biopsy results can often help guide clinical therapy, re-direct clinical work-up and help establish a diagnosis in conjunction with clinical history and lesion characteristics. It is important to remember that lesions have "lives". They may be in an acute, active or chronic phase and therefore in cases of widespread or generalized skin disease, it is imperative to take multiple biopsy samples, ideally three to five 6mm punch biopsy samples. (See below for more details on skin biopsy collection).

Please submit a complete clinical and dermatologic history along with complete lesion description including lesion location. Digital images are a great way to show clinical lesions and can be very helpful to the dermatopathologist when narrowing down differential diagnoses. Photographs can be mailed with the submission or

emailed to pathologyservice@cornell.edu. Our turnaround time is typically about 4 days from the day it is received by the lab. Cases are first reviewed by a pathology resident and dermatopathologist. Most cases will also be reviewed during our weekly dermatopathology rounds with the entire clinical dermatology team and any further opinions are included in an addendum to the initial report. Special histochemical stains (i.e. stains for microbial agents) that are considered important in making a final diagnosis are generally not charged extra as they are considered part of the diagnostic work-up. Additional charges are usually required for immunohistochemistry.

Skin biopsy sample collection: when, where, and how

When to biopsy skin:

1. When lesions are acute and severe.
2. When therapy is associated with significant side effects.
3. When neoplasia is suspected (nodule, chronic non-healing ulcerative lesion).
4. When skin lesions are unusual.
5. When lesions develop while on a course of therapy.
6. When lesions fail to respond to an apparently appropriate course of therapy.

Where to take the biopsy specimens:

1. **Primary lesions** of all types should be sampled first (papular, pustular, nodules, erythema) as they represent the principle pathologic process.

2. As a rule, biopsy **"all" suspect lesions**, particularly when the primary lesions are not easily identifiable. Collect secondary lesions if they represent a significant portion of the disorder (crusts, scale, collarettes, etc.). If crusting is a significant part of the process, collect addition crusts and submit them. Crusts can be useful especially in conditions such as pemphigus foliaceous and dermatophylosis!

3. For depigmenting lesions, collect gray areas or the margin between pigment and non-pigment.

4. For ulcerative lesions, collect the sample on the margin of intact skin extending into the ulcer.

5. For alopecic/hypotrichotic disorders, collect samples from areas that are most alopecia, partially hairless and normal and label separately.

6. Remember that the normal microscopic anatomy of the skin may vary between body locations. For example, skin from the ventrum usually has

fewer adnexal units and therefore fewer hair follicles and smaller sebaceous glands. Thus, if an atrophic condition is suspected, such as an endocrinopathy the ventral abdomen is **NOT** the ideal site for biopsy.

7. Consider complete excision for a solitary nodule.

How to take skin biopsy specimens.
Biopsy techniques.

1. **Biopsy technique.**
   a. NEVER scrub the skin surface, this could remove important diagnostic information. This is NOT a sterile procedure!
   b. Trim hair with scissors or clippers with a #40 blade. Trim above areas where there is abundant scaling and crusting.
   c. Local anesthetic: SQ bleb of 0.5 to 1cc of 2% lidocaine/site. Inject the lidocaine into the subcutis (not the dermis) under or around the biopsy site. (Note: DO NOT use more than 0.5cc total in animals UNDER 10LBS) General anesthesia may be necessary when taking biopsies from mucocutaneous junctions, nasal planum, pawpad and pinna.
   d. Position the punch biopsy instrument over the center of a lesion (preferably) at a site where only abnormal tissue is sampled.
   e. Rotate punch in one direction only, until it sinks into the SQ fat.
   f. Support the plug of tissue from its underside (i.e. do not crush) and cut free with iris scissors. Blot on a gauze to remove excess blood.
   g. Time from removal of the biopsy sample to formalin immersion should be as short as possible (seconds not minutes).
   h. Close with one cruciate or two simple interrupted sutures.
   i. Elliptical and wedge biopsies should be used for larger lesions (nodules, neoplasms), and/or deep (panniculitis) and/or very fragile (big pustules, bullae, vesicles).
   j. Placing the biopsy on a piece of tongue depressor or cardboard to minimize curling during fixation is optimal for thin biopsy specimens (not necessary for full thickness punch biopsies). Allow tissue to dry on the tongue depressor for 30-60 seconds before placing in formalin. NEVER attach the tissue with needles or sutures, this will cause significant artifact. **Keep in mind that the pathologist will transect the specimen through its long axis, symmetrically and perpendicularly to the skin surface.*

2. **General concepts.**
   a. Use a 6 or 8 mm punch biopsy on most cases. Smaller diameter punches (4mm) should only be used when a larger biopsy is technically difficult or could result in visible scarring (pinna, pawpad, near mucocutaneous junction).

b. Secondary bacterial infections are very common and histopathologic reactions obscure the pattern of other diseases. Thus **antibiotics** may be necessary for 2 to 3 weeks prior to biopsy.
c. The effects of **glucocorticoids** can markedly modify reaction patterns and should be stopped for 2-3 weeks prior to biopsy (longer for injectable steroids – 6 weeks!)

3. **Fixative:**
   a. Use 10% neutral buffered formalin at a ratio of 1:10, tissue to formalin. Depending on how cold it gets in your area, during winter months add 1 part 70% ethyl alcohol to 9 parts formalin to prevent tissues from freezing while in transit. Freezing causes tissue artifacts than may hinder the interpretation of the lesions by the pathologist.
   b. Results from "alternative" (formalin-free) fixatives are not as good as fixing tissue in formalin.
   c. Formalin is a proven irritant, a proven sensitizer to delayed hypersensitivity responses and a known carcinogen. It should be handled with caution. If your container leaks in the mail, the post office may send the sample back to you.
   d. Michele's media was once used as a fixative to evaluate for the deposition of immunoglobulins in the skin. Now equivalent results can be obtained with formalin-fixed tissues.

4. **Sample submissions:**
   a. Include in your submission: a brief but complete history, signalment, description of lesions (using proper terminology regarding primary and secondary lesions), location of lesions, presence or absence of pruritus, duration of lesions, pertinent medical history including other diagnostic tests and their results, response to various treatments, current medications patient is taking, and your list of differential diagnoses. DO NOT photocopy and send the patient's medical file.
   b. A **lesion map** and **photographs** of the patient ARE VERY HELPFUL!

5. **Skin biopsy DON'Ts:**
   a. Don't take a punch biopsy within the center of an ulcer.
   b. Avoid using a punch biopsy on large pustules or blisters (bullae) as rotation can shear the roof of the lesion. An excisional biopsy should be used here.
   c. A punch biopsy should not be used to sample neoplastic or inflammatory diseases in the subcutaneous fat (biopsy punches often do not penetrate to a sufficient depth to obtain an adequate sample.
   d. Don't scrub the biopsy site – you may remove important information in the crust.
   e. Don't use dull or previously used punch biopsy instruments.
   f. Don't squeeze the biopsy with forceps.

g. Don't use cautery on small samples such as punch biopsies.
h. Avoid using small (<4mm) punch biopsy instruments.

Dermatology definitions:

Primary lesions

Macule: circumscribed flat area of color change <1cm diameter.
Patch: circumscribed flat area of color change >1cm diameter.
Papule: solid elevated lesion <1cm diameter.
Plaque: flat elevation in skin >1cm.
Pustule: circumscribed elevation of skin containing pus; may be intraepidermal, subepidermal or follicular in location.
Vesicle: sharply circumscribed elevation of epidermis filled with clear fluid, <1cm diameter, intraepidermal or subepidermal
Bulla: sharply circumscribed elevation of epidermis filled with clear fluid, >1cm diameter.
Wheal: sharply circumscribed raised lesion consisting of edema
Nodule: circumscribed solid elevation >1cm in diameter that usually extends into deeper layers of skin
Cyst: epithelium-lined cavity containing fluid or a solid material. It is a smooth, well-circumscribed, fluctuant to solid mass.

Lesions that may be primary or secondary

Alopecia: complete loss of hair
Hypotrichosis: thin hair coat/partial loss of hair
Scale: accumulation of loose fragments of stratum corneum.
Crust: accumulation of dried exudate, serum, pus, blood, cells, or scales adherent to skin surface
Follicular casts: accumulation of keratin and follicular material that adheres to hair shaft extending above surface of follicular ostia.
Comedo: dilated hair follicle filled with cornified cells and sebaceous material
Pigmentary abnormalities: changes in skin color due to a variety of pigments or lack of pigment such as melanin.

Secondary skin lesions

Epidermal collarette: special type if scale arranged in a circular rim of loose keratin flakes or peeling keratin
Scar: area of fibrous tissue that has replaced damaged dermis or subcutaneous tissue
Excoriation: erosion or ulcer caused by scratching, biting, or rubbing
Erosion: shallow epidermal defect that does not penetrate basal laminar zone
Ulcer: break in continuity of epidermis with exposure of underlying dermis

Fissure: linear cleavage into epidermis or through epidermis into the dermis. May be single or multiple, curved, branching or straight
Lichenification: thickening and hardening of skin characterized by exaggeration of superficial skin markings. Often hyperpigmented. Hyperkeratosis is increased thickness of stratum corneum.
Callus: thickened, rough, hyperkeratotic, alopecic, often lichenified plaque

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