

1/26/2008 1:30 PM

Practical Considerations for Submitting Histopathology

Elizabeth M. Whitley, DVM, PhD, Dipl. ACVP
Assistant Professor
Auburn University

Your Pathologist

- Get to know your pathologist. They can add value to your practice as a part of the patient care team!

What Happens to the Tissue You Submit for Histopathology?

- Histopathology Request Form- Day of arrival
- Tissue Sectioning- Day of arrival (unless tissues are not fixed)
- Tissue Processing- Overnight in automatic processor
 - Embedding in paraffin
- Tissues are blocked and sectioned, slides are stained routinely- 2nd day
- Slides delivered to the pathologist- 2nd day
- Pathologist reviews slides, submits request for additional sections or stains
 - “Special stains”
 - Histochemical stains- Gram, Periodic acid Schiff, reticulin, Masson’s trichrome
 - Immunohistochemical staining- Use of an antibody directed to a specific antigen, followed by the application of reagents that allow detection of antibody binding
- Pathologist writes or dictates report and returns it to the referring veterinarian
 - Identification of the tissue being examined
 - Description of the lesion
 - Morphologic Diagnosis
 - Histologic grading of some tumor types
 - Comment

How to Benefit More from Histopathology:

- **Communication with your pathologist**
 - Histopathology request form
 - Signalment-Species, breed, age, sex, name
 - History-lesions, duration, treatments and response to treatment, summary of pertinent laboratory data
 - Your clinical impression- list of differential diagnoses
 - Tissues submitted and anatomic location
 - Results of previous biopsy or biopsy case number
 - Insurance or legal actions anticipated?
 - Verbal communication
 - Follow-up questions
 - Outcome on challenging cases

- **Surgical Biopsy**
 - Incisional vs. excisional biopsy
 - Biopsy site selection
 - Excise the entire lesion and a margin of unaffected tissue, if possible.
 - Select viable tissue for submission, not an area that is necrotic.
 - Gentle tissue handling, especially of very small samples.
 - Avoid crush and stretch artifacts.
 - Don't heat coagulate the biopsy sample by overuse of a laser or electroscalpel.
 - The collection of multiple samples will increase the chance of submitting a sample that is representative of the pathologic process.
 - Mark your surgical margins with India ink or other marking system.
 - Incise thick tissues to increase exposure to formalin, while retaining tissue orientation (don't section all the way through the tissue).
 - Fix tissues in at least a 10X volume of 10% neutral buffered formalin.

- **Challenging Biopsy Samples**
 - Mast cell tumors (MCT)
 - Cutaneous mast cell tumors are most often seen in dogs, and arise from mast cells of the dermis.
 - Mast cell tumors are usually not a diagnostic challenge, but often have infiltrative growth and excisional biopsy is frequently incomplete.
 - Take wide and deep surgical margins if you suspect a mast cell tumor. Consider performing pre-operative fine needle aspiration and cytology.
 - Mark surgical margins with India ink.
 - Osteosarcoma (OSA)
 - Osteosarcoma is a neoplasm comprised of uncontrolled proliferation of osteoblasts.
 - Many osteosarcomas have a zone of reactive (non-neoplastic), proliferating bone at the border that surrounds the neoplastic bony tissue.
 - Collect multiple biopsy samples from deep within the bony lesion. You may want to check your biopsy sites by radiography.
 - Pemphigus foliaceus (PF)
 - Pemphigus foliaceus is a pustular, autoimmune skin disease due to the production of autoantibodies directed to adhesion molecules that attach keratinocytes of the stratified squamous epithelium of the skin's surface and follicles.
 - To diagnose PF histologically, the pathologist looks for detached (rounded up) keratinocytes (aka "acantholytic cells") in subcorneal pustules, along with inflammatory cells (neutrophils and, less commonly, eosinophils) and the absence of bacteria.
 - Pustules that have ruptured and formed a dried crust are not very useful diagnostically.
 - Carefully collect multiple, intact pustules using a biopsy punch.
 - Splenic Hemangiosarcoma

- Hemangiosarcoma is a neoplasm of vascular endothelial cells.
- Hemangiosarcomas of the spleen often are associated with abundant hemorrhage and hematoma formation.
- If you don't submit the entire spleen for histopathology, select multiple samples to submit, choosing areas that are the most "tissue dense".
- Equine sarcoid
 - Sarcoids are composed of proliferating cords of surface epithelium that extend into and overlie sheets of fibroblastic tissue.
 - The pathologist needs to evaluate the surface epithelium, as well as the underlying fibroblastic tissue.
 - Collect incisional biopsy samples from areas that have an intact epithelium and are not ulcerated.