Bone biopsy is essential to differentiate between neoplasia and infectious etiologies (bacterial or fungal). Obtaining diagnostic bone core biopsy samples can be difficult, especially when there is significant periosteal reaction. Oftentimes, the core biopsies we receive are not taken deep enough in the bone and are non-diagnostic. In these cases, we often recommend additional and/or larger bone biopsies from multiple sites and varying depths and/or consultation with a veterinary radiologist. Interpretation of bone biopsy samples ideally should always be performed in conjunction with radiographic interpretation. It is also extremely helpful if the location of the bone biopsy is indicated on the radiograph.

The DCPAH, therefore, now offers a panel that includes decalcification of the bone biopsy, microscopic evaluation of the bone specimen by a board certified pathologist, and radiographic interpretation of the bone lesion by a board certified radiologist for a total fee of $100. The radiologist and pathologist consult with one another and report their interpretations in a single combined report.

Either plain films or digital images are accepted. If digital images are sent, please enclose a CD containing the images. Please indicate on the images where the bone biopsy was taken. This can be done manually on a plain film, electronically on a digital image or even manually on a low quality printed copy of a digital image. The films or CD should be submitted along with the formalin-fixed biopsy specimen and mailed to the DCPAH. Please ensure adequate packaging so that the formalin does not leak onto the films or CD. Please do not mail the samples to the VTH. Entire amputated limbs can also be submitted for this panel but additional processing charges will apply.

**Recommended Guidelines for Obtaining a Diagnostic Bone Biopsy:**

The most common instrument used for bone biopsy is the Jamshidi needle biopsy. Two radiographs, lateral and cranio-caudal, are taken and reviewed. The center of the lesion is located using palpable anatomical landmarks. It is important to biopsy the center of the lesion, as biopsy of the periphery may result in sampling normal reactive bone surrounding the tumor.

The patient is anesthetized. The biopsy area is widely shaved including the predetermined palpable anatomical landmarks, prepared and draped. If a limb lesion is biopsied the leg is shaved 360 degrees and hung. The center of the lesion is localized using the anatomical landmarks. A 2mm stab incision is made in the skin using a number 11 blade. The Jamshidi bone needle, with the stylette in place, is pushed through the soft tissue until it contacts the bone cortex. The stylette is removed and the cannula is advanced through the medullary cavity using a twisting motion. Advancement is stopped before penetrating the far cortex to prevent contamination of uninvolved tissue planes. The bone needle is rocked to allow the sample to break off within the cannula. The instrument is withdrawn and the specimen is pushed out of the base of the cannula by inserting the probe into the cannula tip. Four or five samples are obtained by redirecting the needle through the same stab incision. Material for culture is taken from the samples prior to fixation in formalin.
The DCPAH has begun using the new two tier grading system for canine cutaneous mast cell tumors (MCT) that is of superior consistency and provides more accurate prognostication compared to other grading systems. According to the new system, MCTs are graded as high-grade or low-grade. Grading is not only based on the number of mitoses, but also the presence of multinucleated cells (3 or more nuclei), bizarre nuclei, or karyomegaly. High grade tumors have been shown to be associated with a significantly shorter time to metastasis, MCT associated mortality, and a shorter overall survival time.

The DCPAH has also modified the prognostic panel that is offered for canine MCTs to allow more accurate prediction of clinical behavior and to assist in determining treatment strategies. This updated MCT prognostic panel determines the risk of systemic disease, similar to the previous panel, using immunohistochemical (IHC) labeling for KIT protein (KIT pattern determination) and evaluates cell proliferation using a combination of Ki67 labeling and AgNOR staining (Ki67 x AgNOR index is also included). The panel now includes two PCR tests to check for activating internal tandem duplication (ITD) mutations in the juxtamembrane domain of the c-Kit gene in exon 11, as before, and in exon 8. Mutations in exon 11 have been detected in about 20% of canine cutaneous MCTs; mutations in exon 8 are far less common and occur in less than 3% of MCTs. Based on our research other mutations are so rare that additional screening would not justify the expense.

The complete panel is recommended for all low grade MCTs but each component of the panel can be ordered as an individual test if desired. KIT IHC and PCR testing is recommended for all high grade tumors if therapy with tyrosine kinase inhibitors is considered. Research indicates that cancers caused by c-Kit mutations are highly aggressive, but respond well to tyrosine kinase inhibitor drugs that are now readily available for dogs. See the flow chart on page 3 to aid in therapeutic decision-making.

If no biopsy report has been previously issued for a submitted mass (either by the DCPAH or another diagnostic laboratory), we will first provide a histopathologic description, diagnosis and grading of the submitted MCT. A standard biopsy fee will apply to these cases. If a biopsy report has been issued by another laboratory, please include a copy of this report in the submission. A tissue block (preferred sample), 10 unstained slides all from the same block, or formalin-fixed wet tissue can be submitted for the full panel or for any individual test. Please do not submit tumor margin blocks/slides. PCR is also available on fine needle aspirates (in a tube shipped on ice overnight; you may dilute the sample in 0.2 ml of 0.1 M PBS buffer), but is a much less preferred type of sample.

Please recognize that there is no guarantee that the aspirate contains neoplastic mast cells, or sufficient numbers of mast cells, thereby causing false negative results. Please see the flow chart to the left for pricing information. While there is some association between each independent test, prognoses developed from interpretation of all analyses offer your clients the highest level of certainty.

KIT IHC testing and PCR testing for c-Kit ITD mutations in exons 8 and/or 11 is also available for other canine tumors, including gastrointestinal stromal tumors, melanomas, hemangiosarcomas and germ cell tumors. The cost for each test and the required samples are the same as for MCTs. Additionally, we now also offer PCR to detect a c-Kit ITD in exon 8 in feline mast cell tumors. This test is currently available for $95. The same types of samples can be submitted for this testing as described above. To verify the presence of mast cell...
Flowchart to support therapeutic decision-making based on prognostic parameters for canine cutaneous mast cell tumors. Lymph node cytology is recommended for all MCT prior to excision if there is an accessible regional lymph node (RLN) to guide RLN excision and further staging. Histologic grading should be performed on all surgical biopsy samples followed by additional prognostic tests based on the assigned grade. We strongly recommend further staging for all high grade tumors. Margin evaluation should guide local therapy and interpretation of surgical margins requires knowledge of the methodology used to assess surgical margins. See our margin evaluation option with photographic support in DCPAHealth News, Summer 2010, p. 2 (animalhealth.msu.edu/News/2010_Summer.pdf).

Urinary Bladder Disease: A Challenge By: DCPAH Clinical Pathologists

THE PATIENT: You’re holding your first cup of joe and checking the morning’s appointment schedule. The Smith dog is coming in . . . yet again . . . for frequent passage of red urine! This patient has received two complete series of antimicrobials based upon urinalysis results, but now the owners (and you) might be ready for more extensive diagnostic testing to search for underlying causes such as a calculus or tumor. A diagnostic plan would include many tests from imaging to serum chemistry profile, CBC, urinalysis, and cytology.

THE DIAGNOSTIC CHALLENGE: A complete urinalysis includes dip-stick chemistries, specific gravity, and evaluation of sediment. Evidence of inflammation may include pyuria with possible proteinuria and hematuria. A urine culture with susceptibility testing can direct short-term care of this patient; the goal is to reduce inflammation that can influence cell morphology critical to the diagnosis of malignancy. You’ve seen advertisements for the Veterinary Bladder Tumor Antigen (V-BTA) test. However, this test must be used with caution as many false positives are possible, especially if inflammation, hematuria, proteinuria, or glucosuria are present.1,2

Increased epithelial cells indicate the need for a cytologic evaluation. The most common proliferative cells are epithelial with a range of possible alterations including hyperplasia, dysplasia, or benign to malignant neoplasia. The mere presence of an inflammatory reaction will induce changes in cell morphology with variable evidence of atypia. A cytologic evaluation is often the next step, but urine is a unique fluid with its own challenges and problems.

THE CYTOLOGIC CHALLENGE: Urine is frequently a harsh environment for cells, especially the longer the contact interval. You generally start with a routine urinalysis and progress to a cytologic preparation if indicated. All cytologic preparations of urine will have some evidence of degradation that increases with duration of exposure. Urine cytology samples are best when the bladder has been evacuated recently and concentrated preparations

For more information about research concerning MCT prognosis, please visit our Website at: animalhealth.msu.edu.
(see below) are made as soon as the sample is obtained; unstained, unfixed slides should be sent in a room temperature plastic slide holder.

Most urine samples require some concentration (even with increased cellularity). This can be the sediment sample already utilized for routine evaluation or another cytocentrifugation preparation. It is important to spread cells into a thin layer for optimal cytologic evaluation. Urine sediment slides may be made using a blood-smear technique with a feathered edge or ending abruptly with a band of concentrated cells. With the latter technique, immediately tipping the slide up allows the lead edge of fluid to flow back and form an appropriate thickness for evaluation.

A concentrating device can also be used as was previously described in DCPAHealth News, Fall 2008, p. 3 (animalhealth.msu.edu/News/2008_Fall.pdf). Epithelial cells can then be evaluated for evidence of atypia, but caution is advised when inflammation lingers and/or there is evidence of cell degeneration.

More direct sampling of a mass is also possible via traumatic catheterization. The slight increase in possible complications is balanced by improved potential for cytologic diagnosis of cells with reduced degradation by urine.

Figure 1: Transitional epithelial cells - 50× oil immersion lens, Wright’s stain

A. Traumatic catheterization with well preserved cells (Dx carcinoma)
B. Fresh urine sample with some artifact (suggestive of carcinoma)
C. 3-day-old urine with extensive cell degeneration (nondiagnostic)