

## UC Study Protocols

1. **Collection and evaluation of the urinary tract**
2. **Collection and analysis of stones**
3. **Collection and banking blood for future genetic analyses**

### **Collection and evaluation of the urinary tract (collected from deceased animals)**

Cut the prepuce skin in a circle, leaving approximately 2 inches of prepuce skin around the distal urethra/urethral process, then cut a linear line through skin all the way to the entrance at pelvis, freeing the urethra, but being careful to not cut the penis/urethra. Open abdomen and free up soft tissue in the pelvic canal (this is done by taking a knife or scalpel and staying along the pelvic bones and go in a circle all the way around being careful not to cut the urethra where it bends going across the caudal aspect of the pelvis where it exits). Next find the bladder and trace the ureters cranially toward the kidneys and caudally to the freed up urethra at the pelvic canal/inlet, then pull urethra through pelvic canal into the abdomen, removing the kidneys, ureters, bladder, and full length urethra (including the preputial skin let around the urethral process/distal penis).

The tract should be double bagged (sealable plastic bags are best), placed in a cooler (preferably a boxed styrofoam cooler) along with 2+ frozen ice packs. Ice packs should be wrapped with a paper towel to prevent direct contact with the urinary tract, in order to prevent tissue freezing.

The box should be labeled with a UPS overnight shipping label; which will be provided by Dr. Maggie Highland from the Wisconsin Veterinary Diagnostic Laboratory – please contact Maggie when you need a label (Contact information: [maggie.highland@wvdl.wisc.edu](mailto:maggie.highland@wvdl.wisc.edu) or call 608-213-3025 with questions). The label will be emailed to the participant and will need to be printed out to attach to the box. \*\*Along with the urinary tract, a note should be included in the box providing the following information:

- Owner's name and address
- Study Premises ID and Animal ID
- Date of death/Date tissue was collected

The urinary tract tissue should be left whole and shipped the same day as collection, or as soon as possible. Shipping should occur Monday through Thursday for Tuesday through Friday arrival at the WVDL.

Upon arrival, the tract will be examined as follows:

The urethral length will be measured from (total length, bladder-pelvic flexure, pelvic flexure to the cranial portion of the sigmoid flexure, cranial to caudal points of the sigmoid flexure, caudal point of sigmoid flexure to the distal end of the penis); if still present, the urethral process (“pizzle”) will be measured and evaluated as to whether it remains adhered/attached to the penis or is broken free from the penis.

The tract will be examined for the presence of stones and samples will be collected as follows:

- Cut kidneys in half along the long axis to expose the renal pelvises/recesses
- Palpate the ureters
- Cut open the bladder along the ventral midline into the proximal urethra (just through the sphincter)
- Continue opening the urethra, with scissors, to 2 inches proximal to the point of the pelvic flexure
- Palpate, gently, the remainder of the urethra to check for stones and if present, a small incision is made through the wall of the urethra/penis to remove and collect the stone(s)
- The urethra, from 2 inches proximal to the pelvic flexure extending to the distal end will be fixed whole in 10% neutral buffered formalin.
- Once the tract has fixed for at least 48 hours, but not exceeding 1 week, full thickness cross sections for histologic evaluation will be collected as follows:
  - o At and surrounding the pelvic flexure (1.5 inches proximal to, at the most caudal aspect of the bend/flexure, and 1.5 inches distal to)
  - o Midway between the caudal most aspect for the pelvic flexure and the cranial point of the sigmoid flexure
  - o At the cranial and caudal most points/aspects of the sigmoid flexure
  - o 1.5 inches distal to the caudal point of the sigmoid flexure
  - o Mid-distance between the caudal point of the sigmoid flexure and distal end of the penis
  - o At distal end of the penis just proximal to urethral process
  - o Multiple cross sections of the urethral process
- **In the event that a wether in this study dies of unknown/questionable circumstances, or if there are lesions for which the final diagnosis would benefit from histopathology (light microscopic examination of tissues):** During the necropsy for removal of the specific study tissues (described above), additional tissues may be collected and sent along with the study tissues. Preferably small tissue specimens that are representative of the organs and identified lesions would be placed into formalin for fixation, prior to shipping, in order to best preserve the samples for the best histopathology. If this is not possible, overnight shipment of fresh tissues can also be sent, if formalin is not available. The hope is to provide definitive final diagnosis for the cause of death in all study wethers.

### **Collection and analysis of stones**

All stones should be submitted to Dr. Highland at WVDL. This includes those flushed/removed from live and/or dead animals. If collected from an amputated urethral process, stone can be removed from the tissue and submitted (would be ok to leave within the urethral process if stones are very small with concern of losing them; stones can then be flushed out at WVDL).

Stones should be sent, via USPS, in a plastic tube (such as a plastic red top blood tube) or other clean container/bag placed in a padded envelop. Any stones identified during urinary tract evaluation (described above) will be saved at WVDL. Stone composition will be analyzed at the Minnesota Urolith Center in St. Paul, MN.

Images will be taken, with a ruler in the picture for reference, of the stones and urinary tracts.

Stones will be shipped from WVDL at a minimum monthly to the Urolith Center for analysis.

### **Collection and banking blood (or fresh liver tissue) for future genetic analysis**

In the event that a breed (genetic) association is suspected to be associated with development of urolithiasis, DNA analysis will be performed (*ie.* genome wide association study, or GWAS). Therefore, each animal that is enrolled within the study will have a purple top tube blood sample submitted for DNA banking. This will be done by jugular vein blood collection and placement into a purple top (EDTA) blood tube (preferably 8 mL minimum). This tube should be sent overnight to Dr. Highland at the WVDL by placing in a package (insulated envelop or boxed cooler) and shipping using a label that WVDL will provide. As with the urinary tracts, Dr. Highland can be contacted to provide this label. If blood has not been collected and submitted prior to death of any animal in the study, a sample of liver (1 inch in smallest width at any measurement...*ie.* 1 inch<sup>3</sup>) should be submitted along with the urinary tract. After arriving to the laboratory, blood tubes will be centrifuged (10 min at 2000 x G) and the buffy coat will be collected and placed in a freezer appropriate vial for storing frozen until sent for GWAS analysis (Collaborator: Dr. Brenda Murdoch at University of Idaho).

### **Contact/Shipping Information and Questions for all samples and for sampling procedures:**

Wisconsin Veterinary Diagnostic Laboratory Madison  
Receiving Department  
ATTN: Dr. M. Highland  
445 Easterday Lane  
Madison, WI 53706  
608-213-3025  
[maggie.highland@wvdl.wisc.edu](mailto:maggie.highland@wvdl.wisc.edu)