Transitional cell carcinoma is the most common cancer of the urinary tract in the dog. The most common methods of diagnosis are urinary cytology, suction biopsy of the bladder, and tissue biopsy via cystoscopy or surgery. All of these methods detect the cancer late in the course of disease.

The CADET™ BRAF Mutation Detection Assay is a urine-based DNA test for early detection of canine transitional cell carcinoma. The BRAF mutation is found in 85% of canine transitional cell carcinoma cases and has not been detected in the urine of dogs with other cancers nor in dogs with non-neoplastic inflammatory bladder disease or nonmalignant bladder masses. The test has been shown to detect transitional cell carcinoma in dog up to 4 months prior to development of clinical symptoms. The BRAF Mutation Detection Assay is not affected by hematuria or bacteriuria. Dogs that test positive for the mutation can be monitored during treatment to detect the extent of residual disease.

Each kit contains everything needed to collect and submit the sample. There is a small cardboard box and a white envelope for each kit.

Prepare the urine collection pot
1. Remove collection pot from the clear plastic bag and discard the bag.
2. There is a small volume of clear liquid preservative in the pot. DO NOT DISCARD the liquid.
3. Label the collection pot with the first and last name of the dog in CAPITAL LETTERS

Sample collection
1. Collect urine by free catch or catheter into a separate clean container.

2. **40-60ml of urine is required for each test**
3. If the volume cannot be collected at once, it is ok to collect smaller volumes over 2-3 days.

Packaging and Shipping
1. Screw the top on tightly and place collection pot inside the supplied bag with absorbent pad.
2. Package bag inside small cardboard box provided. Place box inside supplied FedEx bag.
3. Complete green Sample Submission Form.
4. Ship via FedEx. NOTE – cannot be shipped using FedEx drop box
5. Send an email to CADETINBOUND@sentinelbiomedical.com with collection pot number (Dx__________) and FedEx tracking number in the subject header.

Optional Study Participation
1. The bottom half of the green submission form is a consent form and questionnaire for an optional study investigating genetic and environmental factors associated with canine TCC.

Test results are emailed within 2-3 business days of receipt of the sample at Sentinel Biomedical.
Assay Process

Step 1
Urine sample sent in proprietary urine stabilizer/preservative (provided by Sentinel Biomedical) to laboratory for analysis.

Step 2
DNA is isolated from cells shed into the urine.

Step 3
Two fluorescent markers are added to the urine DNA sample. One marker, which is tagged with a green fluorescent dye, matches the normal (non-mutant, or ‘wild type’) BRAF gene sequence. The other, tagged with a blue dye, matches only the mutant BRAF sequence.

Step 4
This mixture is then partitioned into ~20,000 droplets, and the urine DNA in each droplet is allowed to bind to one of the fluorescent markers. Droplets containing urine DNA that binds to the wild type BRAF gene sequence now appear green, while those containing mutant BRAF DNA appear blue.

Step 5:
After binding is complete, each individual droplet is removed from the mixture and scored independently based on its color: green droplets are scored as wild-type, and blue droplets are scored as BRAF mutant. These results are used to calculate the detection threshold and to determine whether a BRAF mutation is detected in the urine DNA sample. If a BRAF mutation is detected, the relative proportion of mutated cells that were shed into the urine can be calculated.

Step 6:
Report is e-mailed

Resources:
1. CADET™ BRAF Mutation Detection Assay Brochure from Sentinel Biomedical.