

**CADET<sup>SM</sup> BRAF  
MUTATION  
DETECTION ASSAY**  
early diagnosis & monitoring of  
canine transitional cell carcinoma  
(TCC)/urothelial carcinoma (UC)



**Early  
Detection.  
Rapid  
Results.**

New research provides  
an opportunity for early  
cancer detection and  
monitoring.



# TCC/UC Overview



## Canine TCC/UC/Bladder Cancer

Transitional cell carcinoma (TCC), also known as urothelial carcinoma (UC), is the most common cancer of the canine urinary tract (1). TCC/UC accounts for an estimated 1-2% of all cancer cases diagnosed in dogs, with an anticipated 80,000+ diagnosed cases this year. It is generally a disease of mid-to-late life, with 95% of cases occurring in dogs age 6 years and older.

While any breed is susceptible to developing TCC/UC, these breeds have higher than average incidence rates: American Eskimo Dog, Australian Cattle Dog, Australian Shepherd, Beagle, Bichon Frise, Border Collie, Parson Russell Terrier, Lhasa Apso, Rat Terrier, Russell Terrier, Scottish Terrier, Shetland Sheepdog, West Highland White Terrier, and Wire Fox Terrier. When combined, these breeds account for over a third of all diagnosed TCC/UC cases in purebred dogs.

TCC/UC affects the bladder, urethra, and kidneys of male and female dogs and also the prostate of males. It is most often detected in the trigone of the bladder, a triangular region of smooth mucosa inside the dorsal wall of the neck of the bladder. Advancing TCC/UC often results in straining to urinate, repeated frequent attempts to urinate, blood in the urine, and bacterial infection. Thickening of the bladder wall can lead to partial or complete obstruction of urine entering the bladder from the ureters, which may lead to kidney failure (2).

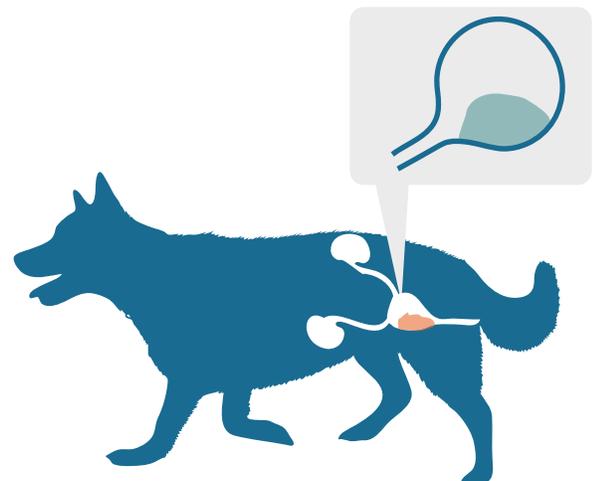
## Today's Diagnosis

Diagnosis of TC/UC is often highly challenging since the symptoms are shared with other much more common urinary tract disorders, including cystitis and prostatitis. In most cases, dogs that present with these non-specific symptoms are initially treated based on the assumption that there is a non-malignant cause, using repeated cycles of antibiotic administration, and sometimes anti-inflammatory medications, over several months. This approach may provide temporary relief of the non-specific symptoms; however the underlying cancer is still enlarging, potentially invading across the bladder wall into the underlying muscle and forming metastases. When repeated treatments fail to fully resolve the non-specific symptoms, the dog is then evaluated specifically for the presence of a TCC/UC, usually via urine cytology, abdominal ultrasound, and/or cystoscopy. Histopathologic evaluation of a tumor biopsy may then be required to confirm the diagnosis and assess muscular invasion.

At the time of diagnosis over 90% of TC/UC cases are intermediate to high-grade invasive tumors (3), and ~20% of cases have already spread to other sites (2). The high predominance of advanced tumors may reflect the prolonged path to diagnosis associated with existing diagnostic strategies.

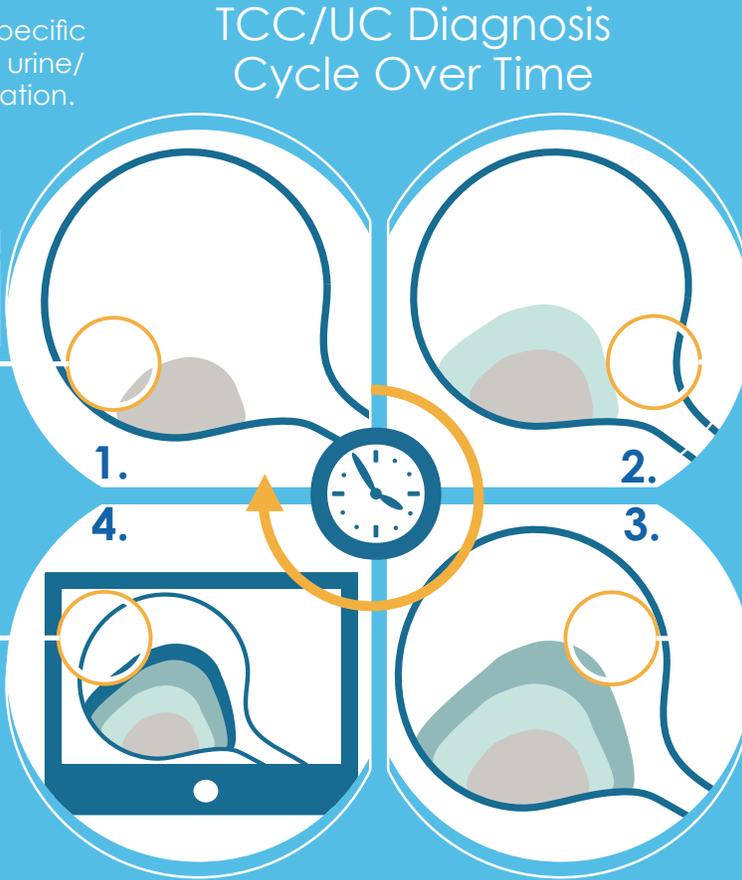
## Introducing the CADET<sup>SM</sup> BRAF Mutation Detection Assay

Sentinel Biomedical now offers a unique DNA-based strategy for early detection of canine TCC/UC. The **CADET<sup>SM</sup> BRAF Mutation Detection Assay** uses a non-invasive, urine-based test that can detect TCC/UC up to four months before clinical symptoms develop. This assay can shorten the path to diagnosis, allowing therapy to be initiated in the earlier stages of the disease, and offering a means to monitor affected dogs during the course of their treatment.



# Conventional Diagnosis

1. Dog shows non-specific symptoms: blood in urine/straining during urination. Antibiotics and/or anti-inflammatory medications administered.



2. Symptoms ease but the tumor continues to enlarge.

4. Symptoms return and ultrasound and/or cystoscopy performed to detect the mass.

3. Antibiotics and/or anti-inflammatory medications administered for a second time. Again, symptoms ease but the tumor continues to enlarge.



## Treatment

Currently, once finally diagnosed, treatment of canine TCC/UC most commonly includes the use of chemotherapy, cyclooxygenase inhibitors, and combinations of these drugs. Where single agent therapy is used, the proportion of dogs entering remission is generally low (<20%), although this is increased to 35–50% with combined chemotherapy and cyclooxygenase inhibitors. While less common than drug based intervention, surgery and radiation therapy are also used where appropriate (1). Regardless of the drug treatment option used, median survival of treated dogs with TCC/UC is currently ~7-9 months.

## The Challenge

Most TCCs/UCs currently go undiagnosed until they are at an advanced clinical stage and so are associated with a guarded or poor prognosis. Detection of the presence of a TCC/UC earlier in the course of disease would allow appropriate intervention sooner, which is expected to improve quality of life and extend survival.

# New Opportunity for Detection



## Research

In two recent independent research studies, a specific mutation in the canine *BRAF* gene was detected in pathology-verified biopsy specimens of canine TCC/UC. A team from North Carolina State University (NC) identified the mutation by comparing the DNA sequence of all dog genes from TCC/UC specimens with those from non-neoplastic tissues (3). A group from the National Institutes of Health (NIH) identified the same mutation by analyzing RNA sequences in tumor tissues (4). Their independent discovery of the same mutation using two different approaches provides robust cross-validation of the data.

The North Carolina State team showed that the *BRAF* mutation was not present in numerous other canine cancers, nor in non-neoplastic bladder tissues, including inflammatory bladder tissue and polyps (5). They developed the CADET<sup>SM</sup> *BRAF* Mutation Detection Assay as a rapid and highly sensitive means to detect TCC/UC cells shed into the urine.

Dr. Breen's laboratory at North Carolina State University has been screening urine specimens from apparently healthy dogs over age 6 years, from breeds with elevated risk of TCC/UC. During this process the research team identified the *BRAF* mutation in several dogs, even though no overt clinical signs of TCC/UC were evident. In each case, subsequent clinical examination of these dogs, followed by high-resolution ultrasound, revealed a very small mass. All subsequently progressed to develop clinical signs of TCC/UC over the following months. This is a very exciting step forward for enabling the earliest detection and treatment of dogs with TCC/UC.

Early detection of a TCC/UC allows earlier confirmation of diagnosis and thus provides more time to treat the cancer and not just the symptoms.

## TECHNICAL DETAILS.

The *BRAF* gene lies on dog chromosome 16. In normal cells, at a specific site in the gene, lies a 'T' nucleotide. In 85% of TCC/UC cases, this nucleotide has mutated into an 'A'. This specific mutation causes an alteration in the gene's amino acid sequence. This results in the production of a defective protein with increased activity. This in turn signals the cells to proliferate abnormally, leading to the development of a malignant tumor.



# Innovative Solution

## CADET<sup>SM</sup> BRAF MUTATION DETECTION ASSAY

The CADET<sup>SM</sup> BRAF Mutation Detection Assay identifies tumor cells carrying a specific mutation in the dog BRAF gene. This mutation is present in urine samples from 85% of all TCC/UC cases, but has not been detected in urine from dogs with numerous other forms of cancer, or with non-malignant bladder inflammation or polyps. The assay can detect as few as 10 mutation-bearing cells in a urine sample, up to four months before any clinical signs associated with the cancer become evident. This enables owners and veterinarians to initiate appropriate treatment very early in the course of the disease, potentially before the mass has become invasive. Additionally, the test allows a sensitive means to monitor affected dogs during the course of their treatment, for therapeutic response and relapse.

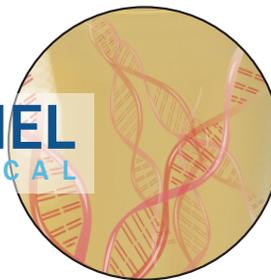
Unlike previous, less discriminatory tests for canine TCC/UC, the CADET<sup>SM</sup> BRAF Mutation Detection Assay is not affected by the presence of blood or bacteria in the urine. Importantly, for cases that have undergone biopsy of a visible mass, there is 100% concordance between the presence of a BRAF mutation in free-catch urine and subsequent pathology-based confirmation of a TCC/UC.

Conversely, the BRAF mutation has not been detected among hundreds of urine samples from dogs that had been diagnosed with non-malignant bladder masses or other forms of cancer. These findings demonstrate that detection of the BRAF mutation in canine urine is a highly specific indicator of the presence of a TCC/UC.

## Assay Process

### Step 1

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

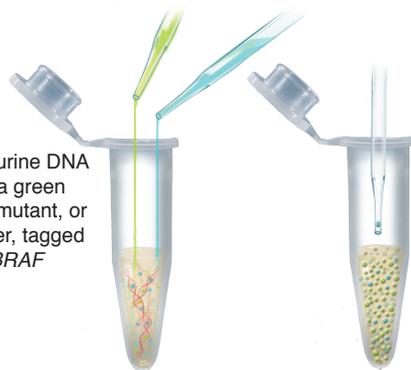


### Step 2

DNA is isolated from cells shed into the urine.

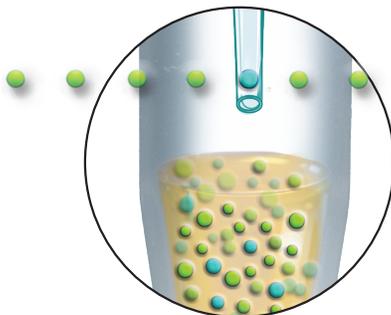
### Step 3

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



PATIENT DETAILS		CLIENT DETAILS	
OWNER:	SENTINEL BIOMEDICAL	CLIENT:	Dr. Katherine Davis
DATE RECEIVED:	06/15/2016	CLIENT ADDRESS:	100 Acacia Avenue, Brentwood, TN 37027
DOB:	06/15/2016	PHONE:	615-251-1111
SEX:	MALE	CLIENT EMAIL:	kat@sentinelbiomedical.com
SPECIES:	DOG (MEDIUM)	DATE RECEIVED:	06/15/2016
SPECIES TYPE:	DOG (MEDIUM)	DATE RECEIVED:	06/15/2016
TEST RECEIVED:	06/15/2016	DATE RECEIVED:	06/15/2016

CLINICAL HISTORY/ANALYSIS SUBMITTED:  
This was returned from the owner with a proprietary method and does not seem to be the presence of a specific mutation in the urine. (BRAF gene)

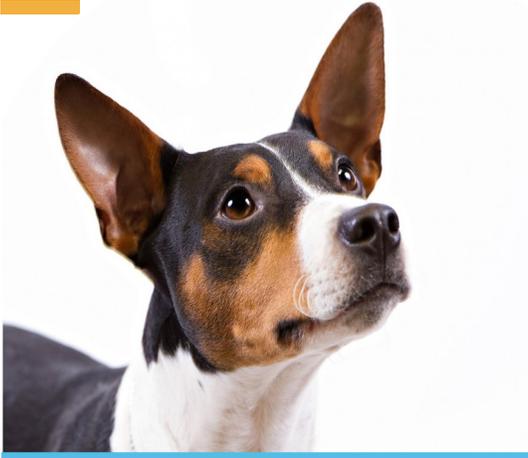
RESULTS:

TEST CONFIRMED	DETECTION STATUS	DETECTION THRESHOLD	NOTES
CADET <sup>SM</sup> BRAF MUTATION	DETECTED	0.00%	

COMMENTS:  
This result of 0% BRAF mutation detected in this urine sample was received. Since the BRAF mutation is generally considered to be a marker for TCC/UC, the results of this test are used to determine the presence of TCC/UC. The CADET<sup>SM</sup> BRAF assay is a highly sensitive and specific test for the presence of TCC/UC. The detection threshold for this assay is 10%.

BACKGROUND INFORMATION:  
The detection threshold for this assay is 10%. The detection threshold for this assay is 10%.

# Test Benefits



## Nationwide Study

Each test includes a questionnaire, allowing owners to be part of a large nationwide research study to investigate the genetic and environmental factors associated with TCC/UC. Breeders, owners, and veterinarians will have access to regular updates from the research program to learn how their dog(s) and patient(s) have contributed.

People share the same kinds of spontaneous cancers and environmental exposures as our dogs. With highly sophisticated analytical tools now available, dogs provide scientists with an ideal, naturally-occurring study population. Early detection tests being developed and offered by Sentinel Biomedical's **CADET<sup>SM</sup>** program not only provide opportunities to potentially extend lifespans of beloved pets, but also offers researchers valuable insight that can be applied to benefit human cancer patients.

### Accessible



- ▶ Non-invasive
- ▶ Easily submit samples to the testing laboratory with a simple free-catch urine test.

### Affordable



- ▶ Avoid costs associated with treating non-specific symptoms
- ▶ Dog owners can direct funds to treating the cancer

### Reliable



- ▶ Forensic-level detection: the CADET<sup>SM</sup> BRAF Mutation Detection Test can detect TCC/UC with as few as ten mutation-bearing cells in the urine sample

### Rapid



- ▶ Results within 2-3 business days
- ▶ Provides more time to optimize treatment strategies

### Benefits all dogs



- ▶ When your clients test their dog(s), they can opt into a nationwide study, helping our research team investigate the genetic and environmental factors associated with TCC/UC. In return, we will send you updates on our research.

Program participation not only helps your patient, but also helps better understand TCC/UC in all dogs.



Our dogs are truly our best friends, in the home and in the fight against cancer.

# Diagnosis/Monitoring

For veterinary professionals, Sentinel Biomedical has developed a special **CADET<sup>SM</sup> BRAF Mutation Detection Assay** service pack to aid the diagnosis of TCC/UC and also for monitoring of BRAF-positive tumors during treatment.

Choose from a single test, a 3-test pack, and a 6-test pack:

- ▶ **SINGLE PACK** - \$159
- ▶ **3-PACK** - \$79 + \$120/test  
when submitted to laboratory
- ▶ **6-PACK** - \$149 + \$110/test  
when submitted to laboratory

8%  
discount

15%  
discount

order directly via  
[www.sentinelbiomedical.com](http://www.sentinelbiomedical.com)

For the single test, the cost covers all fees associated with the test. For the 3-test and 6-test packs, an initial fee is charged for purchase of the service packs. An additional testing fee will be charged to the veterinary practice after each test is completed.

## SERVICE PACK CONTENTS:

All supplies needed to collect and submit urine samples for analysis, sent directly to your clinic.

- FREE shipping to send sample back to the laboratory for testing
- Rapid results in just 2-3 business days from receipt of the urine by the testing laboratory



# CADET<sup>SM</sup>

Canine CAncer DETection

## BRAF MUTATION DETECTION ASSAY

## DIAGNOSIS/MONITORING



## Literature cited

1. Knapp D, McMillan S. Tumors of the urinary system. In: Withrow SJ VD, editor. Withrow and MacEwen's Small Animal Clinical Oncology. St. Louis: Elsevier-Saunders; 2013. p. 572-82.
2. Knapp D, Glickman N, Denicola D, Bonney P, Lin T, Glickman L. Naturally-occurring canine transitional cell carcinoma of the urinary bladder: A relevant model of human invasive bladder cancer. *Urol Oncol.* 2000;5:47-59.
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4. Decker B, Parker HG, Dhawan D, Kwon EM, Karlins E, Davis BW, et al. Homologous Mutation to Human BRAF V600E Is Common in Naturally Occurring Canine Bladder Cancer--Evidence for a Relevant Model System and Urine-Based Diagnostic Test. *Mol Cancer Res.* 2015;13(6):993-1002. doi: 10.1158/1541-7786.MCR-14-0689. PubMed PMID: 25767210; PubMed Central PMCID: PMC4470794.
5. Mochizuki H, Shapiro SG, Breen M. Detection of BRAF Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. *PLoS One.* 2015;10(12):e0144170. doi: 10.1371/journal.pone.0144170. PubMed PMID: 26649430.



## CADET<sup>SM</sup> BRAF MUTATION DETECTION ASSAY

# DETECT. DIAGNOSE. MONITOR.



**DETECT** the presence of TCC/UC up to 4 months (and possibly longer) before any signs of clinical disease become evident. This allows for identification of the disease in its earliest stages.

**DIAGNOSE** TCC/UC quickly. This will allow treatment for the cancer to begin sooner without the delay associated with treating non-specific symptoms.

**MONITOR** the effectiveness of treatment. If the cancer responds to a treatment, the number of cancer cells shed into the urine reduces. The assay may help to determine if the cancer returns after initial therapy, allowing alternative treatment options to be considered.



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