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ISO 13485 CERTIFIED
FM 77123

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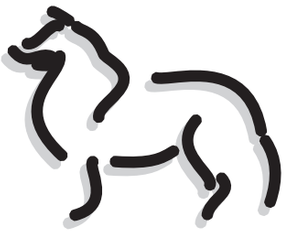
V-BTA™ TEST



6-Test Kit
Cat. No. V664106

15-Test Kit
Cat. No. V664115

A rapid latex agglutination test for the detection of bladder tumor analytes in canine urine as an aid in the diagnosis of bladder cancer.

Caution: This test is intended for veterinary use only and may only be used by or on the order of a veterinarian.

INTENDED USE

The V-BTA rapid latex agglutination test is an *in vitro* device intended for the qualitative detection of bladder tumor analytes in canine urine. Recent research[1] has indicated that the V-BTA test is sensitive for the detection of tumor analytes in canines with TCC of the bladder. The V-BTA test may serve as a useful adjunct to canine TCC diagnosis.

Previously, a version of this test, the BTA® test, had been available in the U.S. indicated for the management of human bladder cancer patients in conjunction with cystoscopy.

SUMMARY AND EXPLANATION OF THE TEST

In dogs, transitional cell carcinoma (TCC) is the most frequently occurring malignancy of the lower urinary tract.[2, 3] Typically, at the time of diagnosis of TCC, the disease is so advanced that the prognosis for dogs is poor.[3] If canine TCC is detected in the early stage of disease, therapies, including surgical resection, and radiation and/or chemotherapy are significantly more effective.[2] Diagnostic tests for the detection of canine TCC include ultrasonography, urinalysis with cytology, fine needle mass aspirate or traumatic catheterization and cytology, radiography with negative or positive contrast cystography, exploratory cystotomy with biopsy, and histopathology and/or cytology.[4,5] Recent research[1] indicates that the V-BTA test, which detects bladder tumor analytes in urine of TCC positive dogs may be a useful adjunct in the detection of canine TCC.

Bladder Tumor Analytes

The bladder tumor analytes detected by the V-BTA test have been isolated and characterized from the urine of some human bladder cancer patients. They are not detected in urine of most normal persons and persons with other diseases. They have been shown to contain high molecular weight (16 - 165 kD) glycoproteins which appear to consist of complexes of basement membrane proteins and in some cases may also contain Immunoglobulin. Three causes for the appearance of bladder tumor analytes in urine of some human bladder cancer patients have been postulated: (1) invasion of the basement membrane (2) production by the tumor itself and (3) a combination of these which may be linked with the body's immune response.

Release of Basement Membrane Complexes

Bladder tumors have been shown to secrete proteolytic enzymes that degrade the basement membrane into fragments of its basic components, e.g., Type IV collagen, fibronectin, laminin and proteoglycans.[6,7,8,9] The loss of basal lamina proteins in the case of bladder cancer leads to the formation of detectable protein complexes in urine, which reflect the tumor's invasive process.[10] These components are discharged into the urine where they combine to form basement membrane complexes. Basement membrane complexes have been detected and characterized in urine as a means to detect tumors in the bladder.

PRINCIPLE OF THE PROCEDURE

The V-BTA test is a latex agglutination assay for the qualitative detection of bladder tumor analytes in urine. Samples of urine are mixed with latex particles coated with human IgG and blocking agents. If the bladder tumor analytes are present in urine at a significant level, they will combine with the latex particles to produce an agglutination reaction. Following the formation of the agglutinates, a visual color change differentiates positives from negatives by use of a specially prepared test strip. Urine samples are considered positive when a clear yellow color is observed above a blue band of agglutinate on the test strip and negative when the test strip is green or green above blue without a yellow color (see "Interpretation of Results").

CONTRAINDICATIONS

- Do not use kit components after expiration date.
- Do not substitute reagents from other V-BTA test kit lots.
- Do not reuse test stations, test strips, or any other disposables used for the test. Discard after single use.
- Do not touch the pad portion of the test strip or use test strips that are damaged.
- Do not use test kits that are delivered damaged or show leakage from reagent vials.
- Do not freeze the V-BTA reagents.
- The positive and negative controls should be clear, pale yellow solutions. The buffer should be clear. If turbidity is evident, the reagents should not be used.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use in dogs.
- Buffer, controls and reagent each contain 0.1 % sodium azide which, if allowed to accumulate, can form explosive compounds in lead and/or copper plumbing. When disposing of buffer, controls, or reagent, flush disposal area with large volumes of water to prevent possible formation of such explosive compounds.
- Human source material used for the preparation of the V-BTA reagent was tested and found to be negative for Hepatitis B Surface Antigen (HBsAg) and for antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), for Human Immunodeficiency Virus Type 2 (HIV-2), and Hepatitis C Virus (HCV). Human source material used for the preparation of the positive control was heat/low pH inactivated. No available test can offer absolute assurance of the absence of infectious agents. Handle these reagents and all materials coming into contact with them as potentially infectious.

LIMITATIONS

False positive test results may be observed in urine samples with 4+ proteinuria, 4+ glucosuria, and >30-40 RBC and/or WBC per hpf. Caution is recommended in interpretation of positive results in cases with pyuria or hematuria.[1]

Results of the V-BTA test should not be interpreted as absolute evidence for the presence or absence of TCC of the bladder. The result from the V-BTA test should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures.

EXPECTED RESULTS

In a recent prospective study[1] conducted at a single institution, sensitivity of the V-BTA test was determined using 20 TCC confirmed canine samples. The overall test sensitivity was 90% (18/20). Overall specificity of the V-BTA test was determined using 45 true negative canine samples including urologic and healthy controls. The overall test specificity was 78% (35/45). The positive predictive value (PPV) was 64% and the negative predictive value (NPV) was 95%.

PERFORMANCE CHARACTERISTICS

INTERFERENCE FACTORS

In a recent canine research study[1] the following parameters were evaluated and exhibited no effect on the V-BTA test:

- Urine pH (range 5.0 - 8.5)
- Specific Gravity (range 1.006 - 1.054)
- Oil Droplets (0 to 4+)
- Bacteriuria (0 to 4+ including positive cultures for *Proteus sp.* and *Escherichia coli*)
- Bilirubinuria, crystalluria (4+ amorphous, 3+ triple phosphate, 2+bilirubin and 4+ ammonium biurate)
- Sperm (4+)
- Epithelial cells (0 to 10 - 20 cells per hpf)
- Granular or hyaline casts (0 - 1 per hpf)

LIMITS OF DETECTION

The sensitivity of the V-BTA test was determined to be approximately 9 μg collagen IV/ml calculated by standard curve analysis. (Human placental collagen IV is used as the standard and control material in the test, and was used in the optimization of the assay).

HIGH DOSE HOOK EFFECT

High dose hook (prozone) effect tests were conducted and results showed that there was no prozone effect up to 4000 μg/ml type IV collagen.

REPRODUCIBILITY

Three lots of V-BTA reagent were used for the reproducibility studies to determine between-day, within-day and lot-to-lot variability. These studies were conducted by testing 14 different collagen IV samples (11 samples were run with 10 replicates, 3 samples were run with 5 replicates) per day for five days. Between laboratory qualitative reproducibility studies were conducted at six laboratories by testing one lot of V-BTA reagent using 14 different samples run 10 times on one day. All reproducibility studies showed near total qualitative agreement with the exception of samples near the cutoff, which is to be expected for qualitative tests.

CONTENTS OF KITS

Buffer Solution
1.0 ml in a dropper bottle. HEPES buffer with 0.1% sodium azide as a preservative.

Negative Control
0.4 ml in a dropper bottle. Saline and glycine buffer with 0.1% sodium azide as a preservative.

Positive Control
0.4 ml in a dropper bottle. Human collagen IV (approximately 60 μg /ml) in saline and glycine buffer with 0.1% sodium azide as a preservative.

V-BTA Reagent
0.7 ml in a screw cap microtube. Polystyrene latex particles (human IgG coated), with blocking agent and 0.1% sodium azide as a preservative.

6-TEST KIT

- Buffer solution
- Negative control solution
- Positive control solution
- V-BTA reagent
- Test stations, 6
- Test strips, 6
- Directions for use
- 35 μl micropipet
- Disposable dropper, 8
- Pipet tips, 16
- Microtubes with caps, 8

15-TEST KIT

- Buffer solution
- Negative control solution
- Positive control solution
- V-BTA reagent
- Test stations, 15
- Test strips, 15
- Directions for use
- 35 μl micropipet
- Disposable dropper, 16
- Pipet tips, 32
- Microtubes with caps,

MATERIALS REQUIRED BUT NOT PROVIDED:

Timer, disposable gloves, urine collection cup, centrifuge, centrifuge tubes.

SPECIMEN COLLECTION, STORAGE AND PREPARATION

Urine that was collected by free catch, catheterization or prior to traumatic catheterization or mass aspiration by antepubic cystocentesis did not interfere with the V-BTA test results in a recent canine study.[1] It should be noted that only a small number of urine samples were collected by catheterization, therefore, the performance of the V-BTA test with urine collected from catheterized dogs has not been established.

Bladder barbotage specimens, serum, plasma or whole blood should not be used. Urine should be collected without preservatives in a clean, dry urine cup. In a recent canine study[1] samples were collected and tested within 48 hours of collection. The stability of canine urine samples beyond 48 hours is unknown. If urine is to be used for other tests, separate out a portion of specimen (a minimum of 2 ml) for this test to avoid contamination. Centrifuge the sample to remove precipitate, transfer supernate to a clean tube and mix before use; discard precipitate. Label the urine sample appropriately. If the urine sample is not to be tested at the time of collection, it should be refrigerated (2-8° C) and brought to room temperature prior to testing. If the urine sample is more than 48 hours old, discard and obtain a fresh sample.

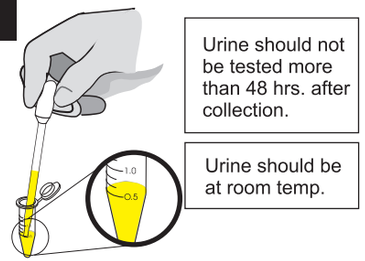
- Do not use paper or foam cups for urine specimen collection or storage.
- Do not test urine specimens that have been heated or frozen.
- The results of testing timed urine collections (24 hour urines) with the V-BTA test has not been investigated.
- In a recent research study,[1] some interference with the test has been observed in urine with elevated levels of leukocytes, protein, glucose, and RBC.
- The effects of Palliative therapy (i.e., piroxicam) on the V-BTA test are unknown.
- The effects of experimental drugs on the V-BTA test are unknown. Dogs treated with experimental or investigational drugs should not be tested until the drug has been fully excreted.
- For trauma to the bladder or urinary tract due to surgery, biopsy, etc., the veterinarian should allow ample time for trauma recovery before using the test.

STORAGE AND STABILITY

- The V-BTA test kit (opened or unopened) is stable until the expiration date indicated on the labeling when the V-BTA reagent and solutions are stored refrigerated (2-8° C). DO NOT FREEZE. All remaining materials (disposables and accessories) can be stored at room temperature (15-30° C). Keep all materials dry.
- Reagents can be used immediately after removal from refrigerated storage. Return reagents to refrigeration after use.

PATIENT TEST PROCEDURE

1 MEASURE URINE



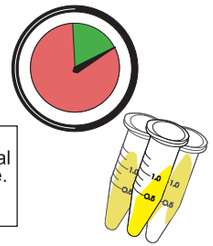
Urine should not be tested more than 48 hrs. after collection.

Urine should be at room temp.

Measure 0.5 ml of **centrifuged** urine into sealable container.

2 ADD BUFFER TO URINE

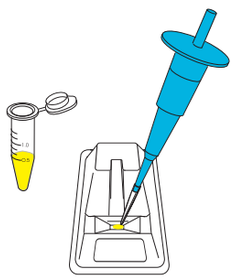
Reagents can be used immediately after removal from refrigerated storage. Return reagents to refrigeration after use.



Add one drop of Buffer to the container.

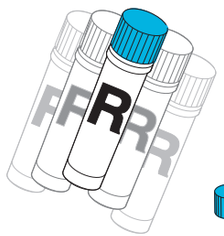
Shake 10 seconds. Test within 30 min.

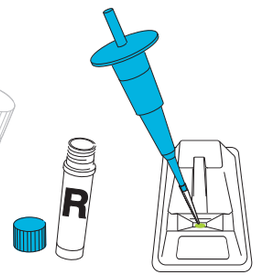
3 ADD URINE TO WELL



Use a micropipet to transfer 35 µl of buffered urine from step 2 to a test station.

4 ADD V-BTA REAGENT TO WELL





Gently shake V-BTA Reagent for 10 seconds.

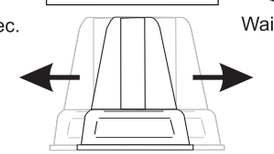
Use a micropipet to transfer 35 µl of V-BTA Reagent to the test station.

5 MIX / WAIT

Mix 10 sec.

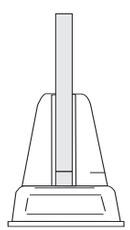
Insufficient mixing is the leading cause of incorrect results.

Wait 20 sec.

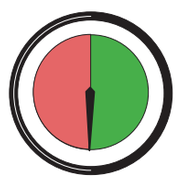


Without splashing, quickly slide test station side-to-side across a flat surface for 10 seconds to mix fluids. Wait 20 seconds but no longer than 5 minutes.

6 ADD TEST STRIP



Lower test strip into well of test station with test pad facing forward.

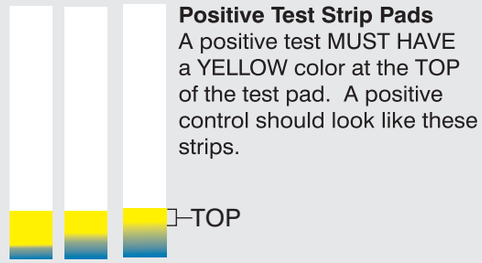


Read after 30 sec. but no longer than 5 minutes.

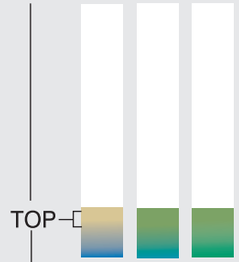
INTERPRETATION OF RESULTS

INTERPRETATION GUIDE

Always compare test strips from urine and controls to picture below.



Positive Test Strip Pads
A positive test MUST HAVE a YELLOW color at the TOP of the test pad. A positive control should look like these strips.



Negative Test Strip Pads
A negative test will have a GREEN or LIGHT GREEN color at the TOP of the test pad. Ignore any blue color at the bottom of the test pad. A negative control should look like these strips.

QUALITY CONTROL

Good laboratory practices recommend the use of appropriate controls. The V-BTA Test includes a positive and a negative control solution. These controls can be used to verify that the V-BTA reagent, test strip and procedure are functioning correctly. The control tests should produce the appropriate result when compared to the Interpretation of Results sample test strips.

CONTROL TEST PROCEDURE:

- Place 2 NEW test stations upright on flat surface. Label one station "+" and one station "-".
- Carefully squeeze ONE drop of negative control (green cap) into the test station well labeled "-" and ONE drop of positive control (red cap) into the other test station well labeled "+".
- Go to step 4 of PATIENT TEST PROCEDURE - "ADD V-BTA REAGENT TO WELL".
- Perform steps 4, 5 and 6 as in PATIENT TEST PROCEDURE

If either the positive or negative control do not give the correct test results, carefully reread the instructions above and repeat the test. Patient results should only be reported if both the negative and positive controls provide the correct results. If problems continue, contact Polymedco Technical Service at 1-800-431-2123.