Evaluation of a bladder tumor antigen test for the diagnosis of lower urinary tract malignancies in dogs

Jean-Philippe H.G. Billet, Dr Vét; Alasdair Hotston Moore, VetMB; Peter E. Holt, BVMS, PhD

Objective—To evaluate the use of a human bladder tumor antigen test for diagnosis of lower urinary tract malignancies in dogs.

Sample Population—Urine samples from dogs without urinary tract abnormalities (n = 18) and from dogs with lower urinary tract neoplasia (20) or nonmalignant urinary tract disease (16).

Procedure—Test results were compared among groups and among 3 observers. The effects of urine pH and specific gravity, degree of hematuria, and storage temperature and time of urine samples on test results were also assessed.

Results—Test sensitivity and specificity were 90 and 94.4%, respectively, for differentiating dogs with lower urinary tract neoplasia from dogs without abnormalities. However, specificity decreased to 35% for differentiating dogs with neoplasia from dogs with nonmalignant urinary tract disease. In dogs with neoplasia, results were significantly affected by degree of hematuria. However, addition of blood to urine from dogs without hematuria had no significant effect on test results. Although intraobserver variation was significant, urine pH, specific gravity, or storage time or temperature had no significant effect on results.

Conclusions and Clinical Relevance—Although this bladder tumor antigen test was sensitive for differentiating dogs with malignancies of the lower urinary tract from dogs without urinary tract disease, it was not specific for differentiating dogs with neoplasia from dogs with other lower urinary tract abnormalities. It cannot, therefore, be recommended as a definitive diagnostic aid for the detection of lower urinary tract malignancies in dogs. (*Am J Vet Res* 2002;63:370–373)

Malignancies of the lower urinary tract (urethral, prostatic, or vesical neoplasia) are rare in dogs. Bladder neoplasms are the most common urinary tract tumors in dogs but still account for < 1% of all neoplasms.[1] Most lower urinary tract tumors in dogs are malignant and are usually transitional cell carcinomas.[2-4] Benign tumors, including fibromas[4,5] and papillomas,[3,4] and nonneoplastic disease (eg, pyogranulomatous polypoid cystitis,[6] eosinophilic granulomas,[7] urethral caruncles,[8] granulomatous urethritis,[9] suppurative prostatitis, benign prostatic hyperplasia, prostatic cysts, benign prostatic hemorrhage), may be mistaken for cancerous lesions. Clinical signs and laboratory findings in dogs with lower urinary tract neoplasia or nonmalignant urinary tract disease are often indicative of nonspecific lower urinary tract disease and include hematuria, stranguria, pollakiuria, and inflammatory cells in urine sediment.[3,9-12]

Lower urinary tract tumors are difficult to diagnose on the basis of results of cytologic examination of urine. Neoplastic cells in the urine sediment are identified in only 30% of dogs with bladder tumors[3] and 40% of dogs with urethral tumors.[11] Examination of tissue specimens obtained by use of a brush technique, suction biopsy, needle biopsy, cystoscopy, or laparotomy are preferred to cytologic examination of urine sediment.[9,10,13,14] Flow cytometric DNA ploidy analysis has been used experimentally in the diagnosis of bladder tumors,[15] and high urine concentrations of basic fibroblast growth factor have been associated with bladder neoplasia,[16] but neither of these tests is commercially available. Contrast radiography is a useful noninvasive diagnostic method for detecting lower urinary tract neoplasia, as is ultrasonography. However, benign masses can be misinterpreted as cancerous.[3-7,10] Cystoscopy and urethroscopy can also aid in the diagnosis of bladder or urethral neoplasia. These techniques require specific fine endoscopes.

Surgery is the preferred treatment for bladder tumors in which complete resection is possible (apical tumor)[3,12,13,17] and for certain types of distal urethral tumors,[2,18] but the prognosis for dogs with most types of lower urinary tract malignancies is poor because of late diagnosis and recurrence after resection. Chemotherapy and piroxicam can be used for palliation or as postoperative adjuvant treatments.[10] Most dogs with lower urinary tract tumors have advanced disease when a diagnosis is made; thus, earlier detection of initial or recurring disease may improve treatment efficacy.

In humans, the **bladder tumor antigen (BTA)** test[a] has proven to be a simple, rapid, and noninvasive diagnostic tool that is superior to cytologic examination of bladder-wash specimens for diagnosing superficial bladder cancer.[19-21] This test has been used for detection of occult or rapid recurrence of bladder transitional cell carcinoma in humans.[19-21] The BTA test appears to be applicable for use in dogs for detection of transi-

Received Apr 30, 2001.
Accepted Aug 27, 2001.
From the Department of Clinical Veterinary Science, Faculty of Medicine, University of Bristol, Langford House, Langford, Bristol BS40 5DU, UK. Dr. Billet's present address is Clinique Vétérinaire de Beaulieu, 18 Blvd Gaëtan Rondeau, 44 200 Nantes, France.
Presented in part at the 10th Annual Scientific Meeting of the European College of Veterinary Surgeons, Velbert, Germany, Jul 6–8, 2001.
Tests evaluated in this study were supplied by Bard Limited, Crawley, UK, and Bion Diagnostic Sciences Inc, Redmond, Wash.
The authors thank colleagues in the Department of Pathology and Microbiology for histologic assistance.
Address correspondence to Dr. Holt.

tional cell carcinoma of the lower urinary tract.[22] The purpose of the study reported here was to determine the sensitivity and specificity of the **veterinary version of the BTA (V-BTA)** test[b] for detection of lower urinary tract malignancies in dogs. The V-BTA test is a latex agglutination assay for the qualitative detection of bladder tumor analytes in urine. It is a colorimetric urine dipstick test.[23] The influences of urine pH, specific gravity, and blood content on test results were also investigated. Interobserver reliability in interpreting test results was assessed, as were the effects of urine sample storage time and temperature.

Materials and Methods

Sample population—Urine samples were collected, with the owners' permission, from 54 client-owned dogs evaluated at the University of Bristol. Twenty dogs had lower urinary tract neoplasia (cancer group), 16 had nonneoplastic disease of the lower urinary tract (abnormal group), and 18 had no biochemical, cytologic, or radiographic evidence of urinary tract disease (control or normal group). In the 20 dogs that comprised the cancer group, results of histologic examination of biopsy or postmortem specimens confirmed the diagnosis of lower urinary tract neoplasia. Five of these dogs had bladder transitional cell carcinoma, 1 had intramural transitional cell carcinoma, 1 had bladder lymphosarcoma, 4 had urethral transitional cell carcinoma, 5 had prostatic adenocarcinoma, 1 had prostatic and urethral transitional cell carcinoma, 1 had prostatic and urethral adenocarcinoma, 1 had bladder and prostatic transitional cell carcinoma, and 1 had bladder and urethral transitional cell carcinoma. The 16 dogs that comprised the abnormal group had clinical signs of urinary tract disease but no evidence of malignancy on the basis of results of contrast radiography, ultrasonography, or laboratory examinations (cytologic examination of urine or fine-needle tissue aspirates and biopsy of diseased tissues). Three dogs in this final group had polypoid cystitis; 1 had polypoid cystitis, benign prostate hemorrhage, and a prostatic cyst; 3 had idiopathic renal hemorrhage; 2 had cystitis; 2 had iatrogenic traumatic cystitis; 1 had urethritis; 1 had hydronephrosis and cystitis; 1 had urethral sphincter mechanism incompetence; 1 had urethral caruncle; and 1 had ureterocele. Two urine samples were collected at a 1-year interval from 1 of these 16 dogs because of recurrence of polypoid cystitis. Thus, 17 samples were collected from 16 dogs in the abnormal group.

V-BTA test protocol—All urine samples were collected by catheterization of the urinary bladder, using a 6-F polyurethane urinary catheter.[c] Not all samples were centrifuged, because centrifugation was not specified in the instruction manual of the test. However, 4 samples from the abnormal group and 1 from the cancer group, all from dogs with gross hematuria, were centrifuged, and only the supernatant was tested. Otherwise, the test was performed according to the manufacturer's instructions.[23] In brief, 0.5 ml of urine was pipetted into a container, 1 drop of buffer was added, and the container was shaken for 10 seconds. Thirty-five microliters of buffered urine and 35 µl of V-BTA reagent were mixed for 10 seconds in a test well. The reagent contains latex particles coated with human IgG and blocking agents. The tip of the paper test strip was immersed in the test well for 30 seconds, and the result was read within 5 minutes. Following the formation of the agglutinates, a color change differentiated positive from negative results. The test was graded from 1 (most positive) to 6 (most negative) according to the manufacturer's colorimetric chart[22]; grades 1 to 3 were considered positive results, and grades 4 to 6 were negative.

Determination of test sensitivity and specificity—Tests were performed on all samples from the cancer (20 samples), normal (18), and abnormal (17) groups. In each case, tests were performed within 2 hours of sample collection and read immediately by the same observer (J-PHGB) who was unaware of diagnostic test results and case identification. To determine whether results of the V-BTA test varied depending on diagnosis, results for the cancer group were compared with those of the normal and abnormal groups by use of Fisher exact tests. Sensitivity (ie, the percentage of positive test results in the cancer group) and specificity (ie, the percentage of negative test results in the normal or abnormal group) were determined.

Determination of urine pH, specific gravity, and blood content—Urinalysis was performed on 46 samples also assessed by use of the V-BTA test: 15 from the cancer group (1 had only pH determined); 17 from the normal group; and 14 from the abnormal group. Urine pH was measured by use of pH paper, and specific gravity was measured by use of a refractometer. Blood content was determined by use of a urine dipstick test[d] and graded 0 for no blood, 1 for trace blood, 2 for 1+ blood, 3 for 2+ blood, and 4 for 3+ blood (ie, maximum degree of hematuria) on the basis of the manufacturer's colorimetric scale. The degree of proteinuria was measured but not recorded, because proteinuria is influenced by hematuria. Relationships between V-BTA test results and urine pH, specific gravity, and degree of hematuria were assessed for all samples and for samples within each group by use of correlation analysis.[e]

Effect of urine blood content on test results—To further investigate the effect of hematuria on test results, fresh canine blood collected for other purposes was added to urine from 6 dogs in the normal group to create 4 dilutions of each sample (10^{-6}, 1.8×10^{-5}, 2.5×10^{-5}, and 5×10^{-5}). Urine without blood corresponded to a urine sample with a grade of 0 (no blood), and the 4 dilutions corresponded to urine samples with trace (grade of 1), 1+ (grade of 2), 2+ (grade of 3), and 3+ (grade of 4) blood on the basis of results of a urine dipstick test.[d] These 30 samples were analyzed by use of the V-BTA test, and the relationship between test results (1 to 6) and urine blood content was assessed by use of correlation analysis.[f]

Determination of interobserver variation—To determine interobserver reliability, 3 observers blinded to group designations read the results of 98 tests (cancer group, n = 36; abnormal group, 25; normal group, 37; all groups included samples retested after different storage times and temperatures) within 5 minutes of each test being performed. Variation among observers was assessed by use of repeated measures ANOVA.[f] Repeated measures ANOVA is more powerful than ordinary ANOVA, because it distinguishes between-subject variability (between rows) from within-subject variability (between columns). If matching is ineffective, however, repeated measures ANOVA may be a less powerful test, because it has fewer degrees of freedom. Thus, for repeated measures ANOVA to be considered preferable to an ordinary ANOVA, matching should be tested for significance.

Effect of storage time and temperature—The effect of storage time and temperature was evaluated, using 24 urine samples. Eight urine samples from the cancer group, 9 from the normal group, and 7 from the abnormal group were divided into 2 after first removing an aliquot for the initial test. Half the sample was stored at 5 C and the other at –20 C. Four tests were performed: the first within 2 hours of collection (no storage), the second after 48 hours at 5 C, the third after 1 week at 5 C, and the fourth after 2 weeks at –20 C. Samples stored at 5 C were warmed to room temperature (approx 24 C), and samples stored at –20 C were

thawed for 6 hours at room temperature prior to testing. Results were compared among storage times and temperatures by use of repeated measures ANOVA.[f]

Statistical analyses—Prior to statistical analyses, data were checked for normal distribution by use of a computer program[e]; all data were normally distributed. For all tests, significance was set at $P \leq 0.05$.

Results

Test sensitivity and specificity—The V-BTA test results were significantly ($P < 0.001$) different in dogs with lower urinary tract neoplasia, compared with the normal group; sensitivity of the test was 90%, and specificity, 94.44% (**Table 1**). However, results did not significantly differ between dogs with neoplasia and dogs with other abnormalities of the lower urinary tract; in this case, specificity was only 35% (**Table 2**). False-positive results were detected for dogs with cystitis (4/5), idiopathic renal hemorrhage (3/3), urethral sphincter mechanism incompetence (1/1), ureterocele (1/1), urethritis (1/1), and urethral caruncle (1/1). False-negative results were detected in 1 dog with prostatic neoplasia and 1 dog with an intramural infiltrative transitional cell carcinoma of the bladder.

Effect of urine pH, specific gravity, and degree of hematuria on test results—When samples from all groups were considered together, urine pH and specific gravity were not significantly correlated, whereas degree of hematuria was significantly correlated ($r = -0.745$; $P < 0.001$) with test results. Within the normal and abnormal groups, none of the 3 variables was correlated with test results, and within the cancer group, only degree of hematuria was significantly correlated ($r = -0.542$; $P = 0.0455$) with test results (**Table 3**). However, when blood was added to urine samples from dogs in the normal group, degree of hematuria was not correlated ($r = -0.2279$) with test results.

Interobserver variation in reading test results—Repeated measures ANOVA revealed a significant variation among results obtained by individual observers ($F = 3.9685$; $P = 0.0205$), with highly significant matching ($F = 62.574$; $P < 0.001$). Mean ± SEM results for each of the observers were as follows: observer A, 3.4 ± 0.20 (range, 1 to 6); observer B, 3.3 ± 0.20 (1 to 6); and observer C, 3.2 ± 0.2 (1 to 6). In 6 of 98 cases, the diagnosis of cancer (ie, positive test result) differed among observers.

Effect of storage time and temperature on test results—Repeated measures ANOVA revealed no significant differences in results obtained for samples analyzed before or after storage ($F = 2.0302$), with highly significant matching ($F = 13.212$; $P < 0.001$). The mean (± SEM) test result for samples analyzed within 2 hours of collection was 3.2 ± 0.44. Results after storage for 48 hours at 5 C, for 1 week at 5 C, and for 2 weeks at –20 C were 3.1 ± 0.41, 3.6 ± 0.41, and 3.6 ± 0.38, respectively. However, storage time and temperature did affect 4 samples that initially yielded positive results and 3 samples that yielded negative results. For 3 of the 4 positive samples, results were negative after storage for 2 weeks at –20 C, whereas for the fourth positive sample, results were negative after storage for any time at either temperature. Two of the 3 negative samples yielded positive results after storage for 2 weeks at –20 C, and the third yielded positive results after 48 hours at 5 C or 2 weeks at –20 C but not after 1 week at 5 C.

Table 1—Results of the veterinary version of the bladder tumor antigen (V-BTA) test for dogs with lower urinary tract neoplasia (cancer) and dogs without evidence of urinary tract abnormalities (normal)

Group	Positive*	Negative†	Total
Cancer	18	2	20
Normal	1	17	18
Total	**19**	**19**	**38**

*Grades 1 to 3 on a 1 to 6 scale were considered positive. †Grades 4 to 6 on a 1 to 6 scale were considered negative.

Table 2—Results of the V-BTA test for dogs with lower urinary tract neoplasia (cancer) and dogs with nonmalignant urinary tract disease (abnormal)

Group	Positive*	Negative†	Total
Cancer	18	2	20
Abnormal	11	6	17
Total	**29**	**8**	**37**

See Table 1 for key.

Table 3—Urinalysis* and V-BTA test results for dogs without evidence of urinary tract disease (normal) and dogs with lower urinary tract neoplasia (cancer) or nonmalignant urinary tract disease (abnormal)

Group (n)	pH	Specific gravity	Blood	V-BTA†
Cancer (15)	7.4 ± 0.22 (6.0–8.4)	1.023 ± 0.0031 (1.010–1.045)	3.6 ± 0.17 (2–4)	1.4 ± 0.22 (1–5)
Normal (17)	7.3 ± 0.19 (5.5–8.4)	1.029 ± 0.0025 (1.011–1.048)	0	5.4 ± 0.23 (2–6)
Abnormal (14)	7.1 ± 0.20 (6.1–8.4)	1.032 ± 0.0020 (1.022–1.042)	2.6 ± 0.48 (0–4)	2.5 ± 0.46 (1–6)

Data reported as mean ± SEM (range).
*pH measured by use of pH paper, specific gravity measured by use of a refractometer, and blood (degree of hematuria) measured by use of a urine dipstick test. †Graded from 1 (most positive) to 6 (most negative). Grades 1 to 3 were considered positive; grades 4 to 6 were negative.

Discussion

The manufacturer of the V-BTA test recommends using it for the detection of transitional cell carcinoma of the bladder.[23] Our results indicate that this test is a sensitive indicator of prostatic, urethral, and bladder tumors in dogs, including tumors other than transitional cell carcinoma. The V-BTA test detects bladder tumor analytes in urine that are produced by the tumor cells themselves or released as a result of tumor invasion of the basement membrane.[23] False-negative results were detected for 1 dog with prostatic cancer and 1 dog with intramural carcinoma of the bladder wall. In these 2 cases, tumor analytes may not have been present in the urine at the time the test was performed because of the extraluminal locations of these tumors.

The V-BTA test was specific for differentiating dogs with lower urinary tract malignancies from dogs without urinary tract abnormalities but was much less spe-

cific for differentiating dogs with neoplasia from dogs with nonmalignant urinary tract disease. Cystitis, urethritis, or hematuria may interfere with test results. The V-BTA test detects protein complexes in urine by use of latex particles coated with human IgG. We found that the test was highly sensitive for detection of lower urinary tract malignancies in dogs, suggesting that human IgG reacted with canine bladder, urethral, and prostatic tumor analytes. However, human IgG may have also reacted with other canine proteins released after tissue damage caused by nonneoplastic disorders.

In the present study, urine pH and specific gravity had no effect on the test results. However, the variations in pH and specific gravity were small (Table 3). Degree of hematuria appeared to influence test results, particularly those of the cancer group. To assess whether blood itself was the factor that interfered with test results within the cancer group, blood was mixed with urine from dogs without abnormalities to create hematuric samples. In contrast to results from a previous study,[22] we found that results of these V-BTA tests were not related to the degree of hematuria. Our results suggest that other factors in urine from dogs with lower urinary tract malignancies and hematuria were responsible for influencing test results during our initial comparisons. Degree of hematuria in dogs with lower urinary tract malignancies may be related to the invasiveness of the tumor itself, and this could explain the relationship we detected between degree of hematuria and test results within the cancer group.

We found significant variation among test results read by 3 different observers. This variation led to a different diagnosis for approximately 6% of the dogs, depending on the observer, and could further reduce the reliability of the test unless repeated tests on the same animal (eg, after treatment) are read by the same observer.

The manufacturer of the V-BTA test recommends that samples should be tested at the time of collection or within 48 hours after refrigeration (2 to 8 C) and states that the stability of canine urine samples beyond 48 hours is unknown and urine samples that have been frozen should not be tested.[23] The lack of significant variation among results for samples tested within 2 hours of collection, after 48 hours and 7 days of refrigeration, or after 14 days of freezing suggests that canine urine samples can be stored and refrigerated for at least 1 week or frozen for at least 2 weeks without interfering with test results. However, in a small number of samples, the test result changed from positive to negative or vice versa after sample storage.

The data reported here suggest that the V-BTA test cannot be used as a definitive diagnostic aid for diagnosing lower urinary tract malignant tumors in dogs. Although the test appears to detect evidence of urinary tract invasion or tissue damage, it did not differentiate between damage resulting from neoplastic disease from that caused by nonneoplastic abnormalities.

[a]Bard BTA test, BARD limited, Crawley, UK.
[b]V-BTA test, Abbott Laboratories Animal Health, Abbott Park, Ill.
[c]Dog catheter, Rocket Medical, Watford, UK.
[d]Multistick, Bayer, Berkshire, UK.
[e]Minitab 8.2, Minitab Inc, Philadelphia, Pa.
[f]Instat 2.01, GraphPad Software, San Diego, Calif.

References

1. Hayes HM. Canine bladder cancer: epidemiologic features. *Am J Epidemiol* 1976;104:673–677.
2. Davies JV, Read HM. Urethral tumours in dogs. *J Small Anim Pract* 1990;31:131–136.
3. Norris AM, Laing EJ, Valli VEO, et al. Canine bladder and urethral tumors: a retrospective study of 115 cases (1980–1985). *J Vet Intern Med* 1992;6:145–153.
4. Schwarz PD, Willer RL. Urinary bladder neoplasia in the dog and cat. *Probl Vet Med* 1989;1:128–140.
5. Esplin DG. Urinary bladder fibromas in dogs: 51 cases (1981–1985). *J Am Vet Med Assoc* 1987;4:440–444.
6. Johnston S, Osborne C, Stevens J. Canine polypoid cystitis. *J Am Vet Med Assoc* 1975;166:1155–1160.
7. Fuentealba IC, Illanes OG. Eosinophilic cystitis in 3 dogs. *Can Vet J* 2000;41:130–131.
8. Bradshaw JM, Brown PJ, Pearson GR, et al. Dysuria associated with urethral caruncle in the dog. *J Comp Pathol* 1996;115:77–84.
9. Moroff SD, Brown BA, Mattiesen DT, et al. Infiltrative urethral disease in female dogs: 41 cases (1980–1987). *J Am Vet Med Assoc* 1991;199:147–251.
10. Phillips BS. Bladder tumors in dogs and cats. *Compend Contin Educ Pract Vet* 1999;21:540–547.
11. Tarvin G, Patnaik A, Greene R. Primary urethral tumors in dogs. *J Am Vet Med Assoc* 1978;172:931–933.
12. Osborne CA, Low DG, Perman V. Neoplasms of the canine and feline urinary bladder: clinical findings, diagnosis, and treatment. *J Am Vet Med Assoc* 1968;152:247–259.
13. Withrow SJ. Tumors of the urinary system. In: Withrow SJ, MacEwen EG, eds. *Small animal clinical oncology*. 2nd ed. Philadelphia: WB Saunders Co, 1996;385–390.
14. Kay ND, Ling GV, Nyland TG, et al. Cytological diagnosis of canine prostatic disease using a urethral brush technique. *J Am Vet Med Assoc* 1989;25:517–526.
15. Clemo FAS, DeNicola DB, Cariton WW, et al. Flow cytometric DNA ploidy analysis in canine transitional cell carcinoma of urinary bladders. *Vet Pathol* 1994;31:207–215.
16. Allen DK, Waters DJ, Knapp DW, et al. High urine concentrations of basic fibroblast growth factor in dogs with bladder cancer. *J Vet Intern Med* 1996;10:231–234.
17. Rocha TA, Mauldin GN, Patnaik AK, et al. Prognostic factors in dogs with urinary bladder carcinoma. *J Vet Intern Med* 2000; 14:486–490.
18. White RN, Davies JV, Gregory SP. Vaginourethroplasty for treatment of urethral obstruction in the bitch. *Vet Surg* 1996;25: 503–510.
19. Sarosdy MF, DeVere White RW, et al. Results of multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. *J Urol* 1995;154:379–384.
20. d'Hallewin MA, Baert L. Initial evaluation of the bladder tumor antigen test in superficial bladder cancer. *J Urol* 1996;155:475–476.
21. Leyh H, Hall R, Mazeman E, et al. Comparison of the BARD test with voided urine and bladder wash cytology in the diagnosis and management of cancer of the bladder. *Urology* 1997;50:49–53.
22. Borjesson DL, Christopher MM, Ling GV. Detection of canine transitional carcinoma using a bladder tumor antigen urine dipstick test. *Vet Clin Pathol* 1999;28:33–38.
23. V-BTA test [package insert]. Abbott Park, Ill: Abbott Laboratories Animal Health, 1999;1–8.