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Evaluation of a Catalase-Based Urine Test for the Detection of Urinary Tract Infection in Dogs and Cats

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Background: Bacterial infection of the urinary tract is a common disorder in dogs and cats. Although microscopic examination of urine sediment is routinely used to screen for infection, this test can lack sensitivity or require expertise. A reliable in-clinic screening test would be a useful adjunct for the identification of dogs and cats with bacterial urinary tract infection (UTI).

Hypothesis: That a catalase-based urine test (Accutest Uriscreen™) is a more sensitive screening test for UTI in dogs and cats than urine microscopic sediment examination.

Animals: One hundred and sixty client-owned dogs and cats.

Methods: Surplus urine from animals presented to a veterinary teaching hospital was used in this prospective observational study. A routine urinalysis, aerobic bacterial culture, and the Uriscreen test were performed on cystocentesis samples. Sensitivity and specificity with 95% confidence intervals and positive and negative likelihood ratios were calculated for Uriscreen and microscopic sediment examination using culture results as the gold standard.

Results: Bacterial culture was positive in 27/165 (16.4%) samples. The sensitivity, specificity, and positive and negative likelihood ratios for the Uriscreen were 89%, 71%, 3.0, and 0.15, respectively. Sensitivity, specificity, and positive and negative likelihood ratios for urine sediment microscopic examination were 78%, 90%, 7.8, and 0.24, respectively.

Conclusions and Clinical Importance: The Uriscreen is a more sensitive screening test for UTI in dogs and cats than sediment examination; however, the urine sediment examination was more specific. A negative Uriscreen result helps exclude UTI; however, urine bacterial culture is still necessary to exclude or confirm UTI in all cases.

Key words: Urinary tract infection; Urine catalase; Uriscreen.

Urinary tract infection (UTI) is a common illness in domestic animals, occurring in up to 14% of dogs[1] and from 3 to 11.8% of cats[2–5] presented for veterinary care. Animals can have overt clinical signs, including pollakiuria, dysuria, stranguria, hematuria, and inappropriate elimination,[6] or the infection might be clinically silent. Occult bacterial UTIs can be associated with underlying disorders such as diabetes mellitus, hyperadrenocorticism, hyperthyroidism, and chronic kidney disease.[7–10]

Urinalysis, including measurement of specific gravity, dipstick testing, and sediment examination, is routinely used to screen for the presence of infection.[11,12] Bacteria can be identified in unstained urine if >10,000 rods/mL or >100,000 cocci/mL are present.[6,13] Urine sediment can be stained with Gram's stain or Wright's stain[14,15] to aid in the detection of bacteria. Infection is also suggested by the presence of pyuria, defined as more than 3–5 leukocytes per high power field.[6] Sediment examination has limited reliability as amorphous debris can mask or mimic bacteria,[16] and failure to identify bacteria does not rule out infection, particularly if urine is dilute.[6] UTI might be present without inflammation in immunosuppressed patients or those with exogenous or endogenous glucocorticoid excess.[6] Additional information could be gathered by dipstick analysis, including esterase testing for detection of leukocytes and nitrite determination for detection of bacteria. However, these tests have poor reliability for the detection of infection in dog and cat urine and should not be used.[6,17]

The Accutest Uriscreen™ (Jant Pharmaceuticals Co)[a] is a rapid, catalase-based test for detection of bacteria and pyuria in urine. Catalases are ubiquitous enzymes that prevent cell oxidative damage by degrading hydrogen peroxide to water and oxygen.[18] These enzymes are found in most bacteria that infect the urinary tract as well as within somatic cells (ie, leukocytes, erythrocytes, bladder and kidney cells). The Uriscreen test uses the catalase reaction to detect bacteriuria, the presence of somatic cells in urine, or

Abbreviations:

CFU	colony-forming unit
CI	confidence intervals
hpf	high power field
lpf	low power field
LR−	negative likelihood ratio
LR+	positive likelihood ratio
RBC	red blood cells
UTI	urinary tract infection
WBC	white blood cells

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both. The expected catalase reaction behavior for common bacterial uropathogens is summarized in Table 1. A positive test is indicative of a significant number of bacteria (>5×10^4 colony forming units per milliliter [CFU/mL]), an abnormally high number of somatic cells (>10 per high power field [hpf]), or both.[19,20] The test takes 2 minutes to perform and does not require specialized training or laboratory equipment.

The Uriscreen test is marketed as a screening test for UTI in human patients, particularly pregnant women and young children,[19–23] and it has been evaluated for the detection of bacteriuria and bacteremia in neonatal calves.[24] The validity of the Uriscreen as a screening test for bacterial UTI in dogs and cats has been investigated[b]; however, to date there are no published reports regarding the application of this test in companion animals. The purpose of this study was to determine the sensitivity and specificity of the Uriscreen test in the identification of UTI in dogs and cats, and to compare its performance with urine sediment examination.

Materials and Methods

Surplus urine from samples collected by cystocentesis from animals presented to a veterinary medical teaching hospital was used in this study. Owner consent was not necessary because the samples were collected for diagnostic reasons by the attending clinicians with no knowledge of the study. Urine samples were submitted in sterile syringes or glass tubes and were held at room temperature until processing within 1 hour of collection. Urinalyses and urine cultures were performed by the Clinical Pathology Laboratory and Clinical Microbiology Laboratory, respectively, at the Texas A&M Veterinary Medical Teaching Hospital. Urine specific gravity was measured by refractometry.[c] Urine dipstick analysis was performed following manufacturer's instructions for the Multistix 10SG.[d] Microscopic sediment examination was performed by trained laboratory technicians on the sediment of 3 mL of urine centrifuged for 5 minutes at $500 \times g$ using the Kova Petters system.[e] Two milliliters of supernatant was removed, and the sediment was resuspended in the remaining 1 mL and placed in a well of the UriSystem DeciSlideon.[f] The number of white blood cells and red blood cells were recorded per hpf (40×), and epithelial cells recorded per low power field (lpf, 10×). Pyuria was defined as ≥3 leukocytes/hpf. Bacteriuria was defined by the presence of any identifiable bacteria on microscopic examination. If bacteria were suspected but not confirmed on the wet mount, smears were prepared, air dried, and stained with Diff-quik for confirmation.[g] Microscopic hematuria was defined as ≥10 red blood cells (RBCs)/hpf. The urine sediment examination was regarded as indicative of infection (active) if either bacteriuria or pyuria were reported and inactive if neither were reported.

Table 1. Expected catalase reaction results for common uropathogens.

Catalase Positive	Catalase Negative
• *Staphylococcus pseudintermedius*	• *Streptococcus* spp.
• *Corynebacterium diptheriae*	• *Enterococcus* spp.
• *Enterobacteriaceae*	
• *Escherichia coli*	
• *Citrobacter* spp.	
• *Enterobacter* spp.	
• *Klebsiella* spp.	
• *Proteus* spp.	
• *Salmonella* spp.	
• *Pseudomonas* spp.	

Urine samples were plated on trypticase soy agar supplemented with 5% sheep's blood (BAP) and MacConkey agar. To quantify bacteria in urine, 2 BAP plates were used for each urine culture. One BAP plate was inoculated with 10 μL of urine (1 : 100 dilution) and 1 BAP was inoculated with 1 μL of urine (1 : 1000 dilution). The inoculated plates were incubated at 35°C in atmospheric air (MacConkey agar) or air supplemented with 5% carbon dioxide (BAP) for up to 3 days and inspected daily for bacterial growth. Bacterial colonies were counted and urinary bacterial concentrations (CFU/mL) were calculated. Urine cultures were considered positive if they grew ≥1000 CFU/mL of 1 bacterial species.

Samples were tested with the Uriscreen within 10 minutes of collection, or, if immediate analysis was not possible, urine was stored at 4°C for up to 4 hours per manufacturer's recommendations in a glass tube before testing. The Uriscreen test was performed in accordance with the manufacturer's instructions by one of the authors (H.K.W.). In brief, an aliquot of urine (1.5–2 mL) was transferred into the test device using a plastic pipette. Each test device contained reagent powder. Four drops of 10% hydrogen peroxide were added to each test device; this was then agitated gently by hand for 5 seconds until the reagent dissolved and the solution turned blue. Each specimen was observed for 2 minutes for foam formation. A test was considered positive if foam was generated and formed a complete and continuous ring or layer on the surface of the solution. A negative result was reported if no foam was noted or if the foam ring was incomplete at the end of 2 minutes (Fig 1).

The sensitivities and specificities (and their respective 95% confidence intervals [CI]) for the identification of infection were calculated for the Uriscreen and for the microscopic sediment examination, using urine culture results as the gold standard. The likelihood ratio of a positive test (LR+) and the likelihood ratio of a negative test (LR−) were calculated similarly using routinely available statistical software.[h] For interpretation of LR+, a value >1 argues for the diagnosis with increasingly larger numbers more convincing for the presence of the disease in question. For interpretation of LR−, values between 0 and 1 argue against the

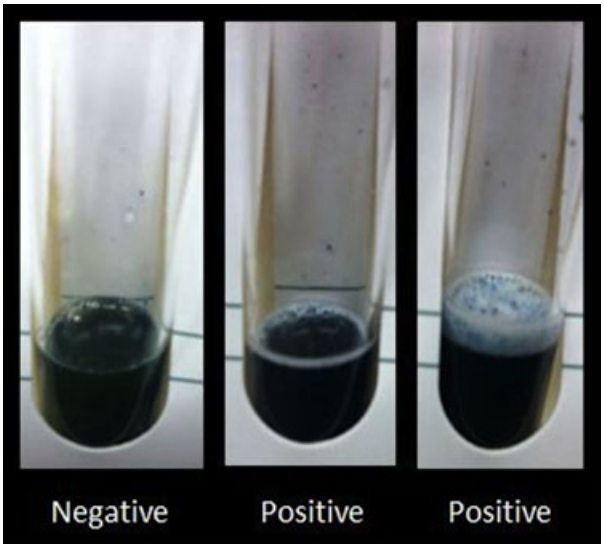

Fig 1. Interpretation of Uriscreen test results.

diagnosis of interest, with values closer to 0 indicating the disease is less likely. An LR+ or LR− equal to 1 indicates that a specific test has limited diagnostic value.

Results

A total of 165 urine samples were evaluated. The samples were collected from 141 dogs (8 intact females, 73 spayed females, 10 intact males, 50 castrated males) and 19 cats (8 spayed females and 11 castrated males). There was 1 cat and 4 dogs for which 2 separate urine samples were available on different occasions. The median age of the dogs was 9 years (range: 0.5–15 years); median age for the cats was 12 years (range: 4–17 years).

Twenty-seven urine samples (16.4%) were positive for infection based on bacterial culture (ie, ≥1000 CFU/mL). *Escherichia coli* was the most common organism identified in 12/27 positive urine cultures.

A total of 64/165 samples had positive results with the Uriscreen test. Twenty-four of the 27 samples with positive culture results were positive with the Uriscreen, giving a sensitivity of 89% (CI: 71–97%). Of the 3 culture positive and Uriscreen negative cases (all from dogs), the first grew 19,000 CFU/mL of alpha-hemolytic *Streptococcus* and had an inactive sediment, the second grew 1000 CFU/mL of *Corynebacterium diptheriae* with an inactive sediment, and the third contained >100,000 CFU/mL *Citrobacter freundii* with an active sediment (1–6 white blood cells [WBC]/hpf and visible bacteria). Two Uriscreen positive cases had growth of catalase-negative bacteria. One was a cat with catalase-negative *Enterococcus* and concurrent pyuria (8–18 WBC/hpf) and one was a dog with catalase negative *Streptococcus* spp. (4800 CFU/mL *Streptococcus canis)* in a mixed infection with >100,000 CFU/mL *Proteus* spp.

Of the 138 samples with no bacterial growth, 98 were negative with the Uriscreen, giving a specificity of 71% (CI: 63–78%). The LR+ was 3.0 and LR− 0.15. When the 20 feline urine samples were analyzed separately, all three of the culture positive samples had a positive Uriscreen test, whereas 6 of 11 samples were Uriscreen positive despite being culture negative.

An active urine sediment was reported in 21 of the 27 culture positive specimens, giving a sensitivity of 78% (CI: 58–91%) for this screening test. In addition, an active urine sediment was found in 13 of the 138 culture negative samples, giving a specificity of 90% (84–95%). All three of the culture positive cases in cats had an active sediment and only 1 of 17 culture negative cases in cats had an active sediment. The LR+ was 7.8 and LR− 0.24 for the presence of an active sediment. Of the 13 samples with an active sediment but a negative culture result, bacteriuria was reported in six. Microscopic hematuria was present in 31/165 urine samples, including 37% (10/27) of culture-positive samples, 38% (15/40) of culture-negative Uriscreen-positive samples, and 6% (6/98) of culture-negative Uriscreen-negative samples. Three cases were reported to have cells on sediment examination consistent with transitional cell carcinoma. All three of these urine samples were Uriscreen positive whereas only 1 of 3 was also culture positive.

The Uriscreen test correctly predicted 4 culture-positive samples in animals with a normal sediment examination, whereas the sediment examination was abnormal in only 1 culture-positive case with a negative Uriscreen (Fig 2). Characteristics of the 2 screening tests compared with urine culture results are summarized in Table 2. Overall, the Uriscreen was a more sensitive test than urine sediment examination for the identification of infection, whereas the urine sediment examination was more specific. When the presence of an active sediment was combined with a positive Uriscreen result, 25/27 samples with a positive culture result were accurately identified (sensitivity 93%; CI: 76–100%).

Discussion

This study evaluated the diagnostic reliability of the Accutest Uriscreen for the identification of UTI in dogs and cats and compared the performance of this test to a standard in-clinic screening method, namely microscopic urine sediment examination. The Uriscreen was found to be more sensitive than the urine sediment examination (89% versus 78%) though less specific (71% versus 90%). The positive and negative likelihood ratios support that whereas finding an active urine sediment on microscopic examination is more specific for UTI than a positive Uriscreen (LR+ 7.8 versus 3.0), the finding of a negative Uriscreen is more likely to indicate absence of infection as compared with an inactive urine sediment (LR− 0.15 versus 0.24). These results are consistent with a previous unpublished study, which found a sensitivity of 92% and specificity of 77% for the Uriscreen.[b] Although false positive results were common with the Uriscreen, UTI is unlikely if the test result is negative.

The most common bacterial isolates from urine samples in dogs and cats are *E. coli*, *Enterococcus* spp., and *Staphylococcus* spp.[9,25–27] False-negative results

Table 2. Results of rapid screening tests compared with urine culture in dogs and cats.

Screening Test (n = 165)	Sensitivity % (CI)	Specificity % (CI)	LR+	LR−
Uriscreen	89 (71–97)	71 (63–78)	3	0.15
Abnormal sediment examination (pyuria, bacteriuria, or both)	78 (58–91)	90 (84–95)	7.8	0.24
Uriscreen + abnormal sediment exam	93 (76–100)	70 (61–77)	3.1	0.1

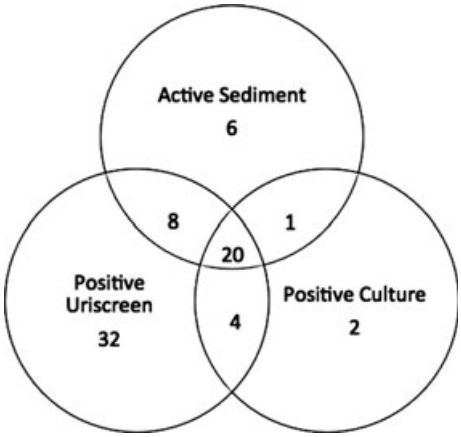

Fig 2. Relationship between Uriscreen results, microscopic sediment examination findings, and bacterial culture in 73 urine samples from dogs and cats with positive results in at least 1 category.

could be caused by bacteria that do not produce catalase such as *Enterococcus* spp. and *Streptococcus* spp. (Table 1). However, the presence of leukocytes in an infected urine sample may trigger a positive catalase reaction irrespective of the causative agent. A positive catalase reaction was reported for the 1 sample in this study with *Enterococcus*; it seems likely that the concurrent pyuria (8–18 WBC/hpf) played a role in generation of the positive Uriscreen result. One sample contained significant numbers of *Streptococcus* spp. (4800 CFU/mL *S. canis)* and was also positive with the Uriscreen. However, this patient had a mixed infection, with >100,000 CFU/mL *Proteus* cultured concurrently, and it is likely that the latter species triggered the positive catalase reaction.

Three of 27 samples (11%) with positive culture results were falsely negative with the Uriscreen. The first of these cases had 19,000 CFU/mL of alpha-hemolytic *Streptococcus* (expected to be catalase negative) and an inactive sediment. This example highlights the limitation of the Uriscreen test with respect to urinary tract infection caused by catalase-negative bacteria. The second had 1000 CFU/mL of *C. diptheriae* and an inactive sediment. Although *C. diptheriae* is expected to be catalase positive, the quantity of bacteria in this sample was below the reported Uriscreen test sensitivity of $>5 \times 10^4$ CFU/mL of urine. The third contained >100,000 CFU/mL *C. freundii* and an active sediment (1–6 WBC/hpf and visible bacteria). As this is a catalase-producing species, the negative Uriscreen result is unexplained. Although every effort was made to perform the test as directed, improper technique may have resulted in a false-negative result in this case, for which not even a partial ring of foam formed.

The relatively low specificity of the Uriscreen makes this a poor test for the definitive diagnosis of UTI in dogs and cats. There are several potential explanations for the frequent false-positive results reported in this study. First, although the Uriscreen may be somewhat less subjective than urine sediment examination, it still requires interpretation by the operator. Care must be taken to differentiate foam from bubbles that form on the surface, often in a ring, because of mixing. The foam is opaque-white versus clear bubbles (Fig 1). A positive test is defined as foam that forms a complete and continuous ring or layer on the surface of the solution. It was noted, however, that the extent of the foam ring or layer varied greatly among "positive" samples. The test may be more reliable if foam forming only a complete layer is considered positive and a continuous ring is regarded as questionable. However, additional study would be necessary to investigate this possibility.

As the Uriscreen is a catalase-based test, positive results may be triggered by the presence of somatic cells in the absence of bacteria. Methods that detect both bacterial and somatic cells yield more false-positive results than methods that detect only 1 cell type.[28,29] Interestingly, 15 out of 40 of the culture-negative Uriscreen positive samples had microscopic hematuria. Red blood cell contamination is not an uncommon consequence of urine sampling by cystocentesis, and could contribute to the high false positive rate seen in this study. Other potential causes of microscopic hematuria that could have been present in the animals included in this study include sterile inflammation from cystoliths, feline interstitial cystitis, and bladder neoplasia. However, hematuria was not consistently associated with a positive Uriscreen result, as 6 culture-negative samples that contained >10 RBC/hpf were Uriscreen negative. This result is somewhat unexpected, as the reported sensitivity of the Uriscreen test for detection of somatic cells is >10 cells/hpf.[19,20]

Another reason for a false-positive Uriscreen result is the presence of renal or bladder cells, as these cells are expected to have catalase reactivity. In this study, 2 false-positive samples contained numerous dysplastic cells consistent with transitional cell carcinoma. Because of the low number of cases, additional study would be required to investigate the likelihood of a false-positive catalase test in samples from patients with transitional cell carcinoma of the urinary bladder.

One potential limitation to this study was the lack of concurrent information regarding clinical index of suspicion for UTI. The attending clinicians submitted urine samples, and information regarding patient status was not available to the authors. A urine sample obtained by cystocentesis with bacterial growth <1000 CFU/mL may be considered clinically significant if the patient is showing compatible clinical signs.[6,30] The specificity of the test may have been improved if only urine from animals with a high clinical suspicion of infection, ie, clinical signs or underlying predisposing conditions, was investigated.

One additional limitation of this study is the small sample size, with only 27 culture positive urine samples included in the 165 samples tested. One reason for the limited number of infected samples was that only urine in excess of that needed for clinical evaluation was

tested with the Uriscreen. As animals with UTI are often pollakiuric, surplus urine was less likely to be available from affected individuals. Despite this apparent limitation, the study size is consistent with several similar studies in humans[20–23] and other veterinary studies investigating urinary tract infections.[7,9,31–34] This requirement of up to 2 mL of additional urine in order to perform the Uriscreen test has the potential to be problematic in pollakiuric patients, and may limit its use in this population.

A rapid and reliable in-clinic screening test is helpful for the identification of bacterial UTI. The results of this study support the use of the Uriscreen test in dogs and cats as an adjunct to routine sediment examination and microbiologic culture. A negative Uriscreen result helps exclude the possibility of UTI, however, because of 11% false-negative and 24% false-positive test results, urine bacterial culture is still necessary to exclude or confirm UTI in all cases.

Footnotes

[a] Accutest Uriscreen, Jant Pharmaceuticals, Mundelein, IL
[b] Bellino C, Farca AM, Abate O. The validity of a catalase test for early detection of urinary tract infection (UTI) in dogs and cats. The 12th ECVIM-CA/ESVIM Congress, 2002. Available at: http://www.vin.com/Members/Proceedings/Proceedings.plx?CID=ecvim2002&PID=pr02291&O=VIN (accessed October 15, 2012)
[c] ATAGO Automatic and water resistant refractometer, ATAGO U.S.A., Inc, Bellevue, WA
[d] Siemens Healthcare Diagnostics Inc, Tarrytown, NY
[e] Hycor Biomedical Inc, Garden Grove, CA
[f] Fisher Healthcare, Pittsburgh, PA
[g] Siemens Healthcare Diagnostics Inc
[h] GraphPad Prism Version 5, La Jolla, CA

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Conflict of Interest: Authors disclose no conflict of interest.

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