

Diagnostic accuracy of a point-of-care test using voided urine samples for detection of bacteriuria in dogs with signs of lower urinary tract disease

David C. Grant  | Michael T. Nappier | Virginia Kiefer Corrigan

Virginia-Maryland Regional College of
 Veterinary Medicine, Blacksburg, Virginia

Correspondence

David C. Grant, Virginia-Maryland Regional
 College of Veterinary Medicine, Duck Pond
 Dr., Blacksburg, VA.
 Email: dgrant@vt.edu

Abstract

Background: Bacterial urine culture is recommended in dogs suspected of having urinary tract infection (UTI), but there is expense and delay in obtaining such results.

Hypothesis/Objective: To determine the diagnostic performance of a rapid immunoassay (RIA) dipstick for detection of bacteriuria using voided urine from dogs with clinical signs of lower UTI.

Animals: Twenty-four client-owned dogs.

Methods: Voided urine was collected and the RIA performed within 30 minutes. Urine collected by cystocentesis was submitted for aerobic urine culture. McNemar's test and kappa coefficient were calculated to determine agreement between the 2 tests.

Results: Nine of 21 dogs (43%) had UTI verified by aerobic urine culture. There was 1 false-negative and no false-positive RIA results. Sensitivity, specificity, positive predictive value, and negative predictive value of the RIA were 89%, 100%, 100%, and 92%, respectively.

Conclusions and Clinical Importance: This RIA is promising for correctly identifying whether or not voided urine samples from dogs with lower urinary tract clinical signs have true bacteriuria in a rapid, inexpensive manner. Additional patients should be enrolled in a similar study to determine if diagnostic performance is robust in a large population.

KEYWORDS

bladder, cystitis, infection, urinary tract, urine culture

1 | INTRODUCTION

Definitive diagnosis of urinary tract infection (UTI) requires bacteriologic culture of urine or urinary tissue. The International Society for Companion Animal Infectious Diseases recommends culture of urine

samples from all dogs suspected of having UTI.¹ Cystocentesis is the recommended collection method for samples to be cultured because this method bypasses the distal urethra, which is inhabited by commensal bacteria even in healthy dogs.^{1,2} Urine culture with susceptibility testing is recommended to discriminate infection from noninfectious diseases that mimic UTI, thereby directing optimal treatment and promoting antimicrobial stewardship. There is concern that indiscriminant use of antimicrobials contributes to bacterial antimicrobial resistance.¹

Abbreviations: cfu, colony-forming units; CI, confidence intervals; NPV, negative predictive values; PPV, positive predictive values; RIA, rapid immunoassay dipstick; UTI, urinary tract infection; VITALS, Virginia Tech Animal Laboratory Services.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals LLC. on behalf of the American College of Veterinary Internal Medicine.

Despite these recommendations, many veterinarians collect voided urine for urinalysis even when dogs have signs consistent with UTI. Voiding collection likely is chosen because it is simple and can be performed by dog owners, and it is convenient, pain-free, restraint-free, and cannot introduce iatrogenic hemorrhage into the sample, all of which are potential issues with cystocentesis. In some instances, such as patients with thrombocytopenia or those receiving anticoagulant medications, cystocentesis actually may be contraindicated. Veterinarians report cost as the main barrier to performing culture and susceptibility testing.³ This decreases the necessity for collection by cystocentesis and may be a reason why cystocentesis is not selected for collection routinely by all veterinarians. Rapid, less expensive point-of-care tests for bacteriuria have been compared with urine culture. One study assessed unstained and modified Wright-stained urine sediment slides.⁴ The unstained and modified Wright-stained slides had sensitivities of 82.4% and 93.2%, specificities of 76.4% and 99.0%, positive predictive values (PPV) of 40.1% and 94.5%, and negative predictive values (NPV) of 95.8% and 98.7%, respectively. To achieve these results required that all slides be reviewed by a single board-certified clinical pathologist and urine be collected by cystocentesis. Other tests include compartmented bacteriologic, urine dipstick paddle, and rapid urine catalase tests.^{5,6,7} The former 2 tests required a minimum of 24 hours to complete, and all 3 were performed on urine collected by cystocentesis or urinary catheterization to achieve good performance.

Recently, another study reported use of a point-of-care rapid immunoassay (RIA) urine dipstick (RapidBacVet, Silver Lake Research Corporation, Azusa, California) for detection of bacteriuria in 200 urine samples from dogs.⁸ Of these, 92% were collected by cystocentesis, 6% by urinary catheterization, and only 2% (4 samples) by voiding. The sensitivity, specificity, PPV, and NPV were 71.7%, 100%, 100%, and 91%, respectively. A major limitation of the study was that no mention was made of whether or not dogs had lower urinary tract signs, including suspicion of UTI. The RIA was performed by trained laboratory personnel at a diagnostic laboratory. However, the test does not require specialized training. The RIA takes <20 minutes to complete and is less costly than commercial culture.

We were interested to determine if this RIA would perform well in a typical general practice setting wherein licensed veterinary technicians perform the test on voided urine samples at the time of the patient appointment for evaluation of lower urinary tract clinical signs. We hypothesized, in this setting, positive and negative RIA results would correlate well with positive and negative bacterial growth, respectively, on aerobic bacterial culture of urine samples collected by cystocentesis.

2 | MATERIALS AND METHODS

Ours was a prospective study performed at the Virginia-Maryland College of Veterinary Medicine performed between January and December of 2017. Dog owners were offered enrollment with signed consent if their veterinarian recommend aerobic bacterial urine culture because of the presence of clinical signs of lower urinary tract

disease. Clinical signs had to include at least 1 of the following: pollakiuria, stranguria, hematuria, periuria, excessively licking of the genital area, and urinary incontinence. There were no exclusion criteria, including recent administration of antimicrobials. The RIA^d was performed by licensed veterinary technicians within 30 minutes after collection of voided urine, and the remainder of the sample was submitted for urinalysis performed by medical technologists of the Virginia Tech Animal Laboratory Services (VITALS) within 30 minutes. Neither cleansing nor an attempt to collect urine at midstream were done. Urine also was collected by antepubic cystocentesis within ± 4 hours of the voided sample (no medications were administered to dogs in the interim) and was plated for aerobic bacterial urine culture by VITALS within 30 minutes of collection. Licensed veterinary technicians and medical technologists were blinded to the results of the test performed by each other.

The RIA was performed according to the kit instructions (RapidBacVet, Silver Lake Research Corporation). The test utilizes a cocktail of monoclonal antibodies targeting a panel of bacterial surface proteins (personal communication from Erik Serrao, Vice President, Sales & Business Development, Silver Lake Research Corporation). First, 150 μ L of assay buffer was added to the included test vial, which then was swirled occasionally during a 2-minute waiting period at room temperature. Subsequently, 150 μ L of urine was added to each vial, swirled to mix, and allowed to stand for 5 minutes at room temperature. Then, the included test strip was placed into the test vial, and remained there for 10 minutes. The strip was removed, and results interpreted as positive, according to the instructions, if either lines 1 and 2 (gram-negative bacteria) or only line 2 (indeterminate gram type) appeared, and negative if only the control line appeared.

For aerobic bacterial urine culture, a 10- μ L calibrated loop was used to streak urine onto 5% sheep blood agar, which then was incubated for 48 hours at 35°C in 7.2% CO₂ and onto MacConkey agar which then was incubated at 35°C in room air. After incubation, any growth was sampled and identified using matrix-assisted laser desorption/ionization time of flight mass spectroscopy, and growth quantified. Any growth was considered a positive result indicative of bacteriuria and a UTI because all dogs had lower urinary tract clinical signs that could be caused by UTI. The result of culture was the criterion-referenced result to which the RIA was compared.

Sample size calculation was performed using the following assumptions: prevalence of UTI 20%,⁸ 80% power, and a *P* value of .05. This calculation indicated a sample size of 60 (which includes 12 subjects with positive urine cultures) would achieve 86% power to detect a change in sensitivity from 0.5 to 0.89 using a 2-sided binomial test and 100% power to detect a change in specificity from 0.5 to 0.99 using a 2-sided binomial test. Thus, our goal was to enroll at least 60 dogs. Sensitivity, specificity, PPV, and NPV for the RIA, using the urine culture result as the definitive result, and 95% confidence intervals (CI) were calculated using standard equations. A Chi-squared (McNemar's) test was performed to determine the association between the RIA and aerobic bacterial culture results. To assess agreement between urine aerobic bacterial culture results and RIA results, a simple kappa statistic (κ) and 95% CI calculated. *P* values of <.05 were considered significant.

3 | RESULTS

Twenty-six dogs were enrolled in the study. Five were eliminated. The 1st was found to have a bladder mass after the RIA was performed but before submission of the urine culture, and the veterinarian chose not to submit a sample for culture. The 2nd dog was eliminated because its RIA test was found to have been performed on an expired RIA test strip. Medical record review of the other 3 dogs indicated they did not have any lower urinary tract clinical signs, and should not have been enrolled. Thus, the analysis included 21 urine samples collected from 21 separate dogs.

Clinical signs included pollakiuria (11 dogs), periuria,⁸ urinary incontinence,⁶ and stranguria.⁴ The sex of dogs with positive urine cultures included 7 spayed females, 1 intact female, and 1 neutered male dog. The sex of the dogs with negative urine cultures included 8 spayed females and 4 neutered males. No dogs had recently been treated with or were receiving antimicrobials at the time of testing.

There were 8/21 positive RIA tests, all of which had positive urine cultures, and thus were classified as true positives. The bacterial colony counts were >100 000 colony-forming units (cfu/ml) in urine from 7 of these dogs and 200 cfu/mL in urine from 1 dog. The bacterial species was *Escherichia coli* in 5 patients, *Klebsiella* spp. in 2 patients, and a mix of *E. coli*, *Enterococcus faecalis*, and *Staphylococcus pseudintermedius* in 1 patient. One of 21 RIA tests was negative with a positive culture and was classified as a false negative. This urine sample lacked pyuria, but did have rarely seen microscopic bacteria. The urine culture grew >100 000 cfu/mL *E. faecalis*. This dog had not received any medication other than phenylpropanolamine for several months before testing. All remaining RIA tests were negative with negative urine cultures, and thus were classified as true negatives.

The sensitivity of the RIA was 89% (CI, 52%-100%), specificity 100% (CI, 74%-100%), PPV 100% (CI, 63%-100%), and NPV 92% (CI, 64%-100%). Results of urine culture and RIA were not independent ($P = .32$). There was strong agreement between urine culture results and RIA results; κ was 0.90 (95% CI, 0.71-1.0).

4 | DISCUSSION

We investigated the performance of an RIA for detection of bacteriuria using voided urine from dogs with clinical signs of lower urinary tract disease. Diagnostic performance was excellent, especially considering urine samples were collected by voiding without any patient preparation such as genital wiping or irrigation. Collection of urine by voiding is not only common in veterinary practices, but it is easy for veterinary staff and dog owners alike to perform, making it convenient.

The conceptual benefit of a test such as the RIA used in our study is to rapidly screen patients with lower urinary tract signs to determine if there is reason to perform a urine culture. As such, high sensitivity is desired such that all animals that truly have bacterial UTI would test positive, and have urine culture recommended. The RIA sensitivity was 89%, which is good, but along with PPV and NPV had a wide CI due, to some extent, to the small population used.

Additional research should be performed to determine sensitivity in a larger population. The sensitivity, specificity, PPV, and NPV of the RIA are the same or higher than those reported for other screening tests, including a compartmented bacteriologic culture, urine dipstick paddle, and catalase tests performed on urine collected by cystocentesis. The RIA had higher specificity and rapid results and used voided urine.^{5,6,7} The high NPV should decrease the recommendation for aerobic urine culture and susceptibility testing, thus saving the client money, but also should prevent unnecessary treatment with antibiotics. A negative RIA result also allows the veterinarian to quickly focus diagnostic testing on causes of lower urinary tract clinical signs other than bacterial UTI. The 100% PPV makes it clear which dogs should undergo cystocentesis and submission of that sample for culture and susceptibility.

For dogs with a positive RIA, the RIA may have 2 disadvantages. First, the RIA will result in an additional cost beyond aerobic urine culture. Second, if the RIA was performed on a voided sample dropped off at a veterinary practice, the owner would need to return again for cystocentesis and culture. However, in our population, > 50% of dogs that had lower urinary tract clinical signs had negative urine cultures, and thus clients to whom a urine culture was recommended (based on their dog's clinical signs) would save money by using the RIA dipstick to exclude bacterial infection as the diagnosis. We did not evaluate correlation of cytologic pyuria, hematuria, or bacteriuria with urine culture results. A grading scheme utilizing these cytologic features in addition to the RIA might be superior to any individual test. There were no false-positive RIA results, suggesting that presence of distal urethral commensal bacteria may not influence the result of this point-of-care RIA. We did not utilize the gram classification feature of the RIA.

The sensitivity, specificity, PPV, and NPV of the point-of-care RIA for the detection of bacteriuria in our study were very similar to those reported for identification of bacteriuria using modified Wright-stained samples collected by cystocentesis and examined by a board-certified clinical pathologist.⁴ The performance characteristics of the RIA were very similar to those reported when this same RIA was used to identify bacteriuria in samples predominantly collected by cystocentesis, but possibly with higher sensitivity (89% vs. 71.7%).⁸ However, direct comparison of our results and these 2 studies is inappropriate. Those studies included samples regardless of whether clinical signs of UTI were present, included multiple samples from the same dogs, and did not report whether dogs were being treated with antimicrobials at the time of testing. Diagnosis of UTI in those studies was based merely on culture detection of >1000 cfu/mL.⁸ Doing so is not an appropriate way to diagnose UTI because subclinical bacteriuria can occur in dogs and is distinct from UTI. In healthy and in morbidly obese dogs, the prevalence of subclinical bacteriuria is 10% and 13%, respectively.^{9,10} Our study included only dogs with lower urinary tract clinical signs commonly associated with bacterial UTI. Although the previous studies^{4,8} included several hundred urine samples each, thus increasing statistical validity, both had lower prevalence of positive urine cultures (16%⁴ and 28%⁸) than the 43% prevalence in our study. Both PPV and NPV are affected by prevalence, further limiting comparison of these results to our study.

Our study utilized a small population and as a result is underpowered with regard to determining the true sensitivity. A similar study should be repeated with a population at least 2 times larger than ours before drawing conclusions on the diagnostic performance of this RIA using voided urine. Other aspects of the RIA, including precision and effects of medications on the results, should be investigated.

In conclusion, this simple point-of-care RIA test can be performed in-office, rapidly, at low-cost, and without specialized training. It may offer monetary savings to dog owners and facilitate patient diagnosis and treatment. We believe performance of this RIA on voided urine is promising for correctly identifying presence or absence of true bacteriuria in dogs with lower urinary tract clinical signs, thus clarifying which patients should have urine culture performed. Additional patients should be enrolled in a similar study to determine if diagnostic performance is robust in a large population.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Virginia Polytechnic Institute and State University IACUC, number 16-238.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

David C. Grant  <https://orcid.org/0000-0002-7279-380X>

REFERENCES

1. Weese JS, Blondeau J, Boothe D, et al. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. *Vet J*. 2019;247:8-25.
2. Ling GV, Ruby AL. Aerobic bacterial flora of the prepuce, urethra, and vagina of normal adult dogs. *Am J Vet Res*. 1978;39:695-698.
3. Fowler H, Davis MA, Perkins A, et al. A survey of veterinary antimicrobial prescribing practices, Washington State 2015. *Vet Rec*. 2016;179:651.
4. Swenson CL, Boisvert AM, Kruger JM, Gibbons-Burgener SN. Evaluation of modified Wright-staining of urine sediment as a method for accurate detection of bacteriuria in dogs. *J Am Vet Med Assoc*. 2004; 224:1282-1289.
5. Olin SJ, Bartges JW, Jones RD, Bemis DA. Diagnostic accuracy of a point-of-care urine bacteriologic culture test in dogs. *J Am Vet Med Assoc*. 2013;243:1719-1725.
6. Ybarra WL, Sykes JE, Wang Y, Byrne BA, Westropp JL. Performance of a veterinary urine dipstick paddle system for diagnosis and identification of urinary tract infections in dogs and cats. *J Am Vet Med Assoc*. 2014;244:814-819.
7. Kvitko-White HL, Cook AK, Nabbity MB, et al. Evaluation of a catalase-based urine test for the detection of urinary tract infection in dogs and cats. *J Vet Intern Med*. 2013;27:1379-1384.
8. Jacob ME, Crowell MD, Fauls MB, Griffith EH, Ferris KK. Diagnostic accuracy of a rapid immunoassay for point of-care detection of urinary tract infection in dogs. *Am J Vet Res*. 2016;77:162-166.
9. Wan SY, Hartmann FA, Jooss MK, Viviano KR. Prevalence and clinical outcome of subclinical bacteriuria in female dogs. *J Am Vet Med Assoc*. 2014;245:106-112.
10. Wynn SG, Witzel AL, Bartges JW, Moyers TS, Kirk CA. Prevalence of asymptomatic urinary tract infections in morbidly obese dogs. *PeerJ*. 2016;4:e1711.

How to cite this article: Grant DC, Nappier MT, Corrigan VK. Diagnostic accuracy of a point-of-care test using voided urine samples for detection of bacteriuria in dogs with signs of lower urinary tract disease. *J Vet Intern Med*. 2021;35: 993-996. <https://doi.org/10.1111/jvim.16040>