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Point-of-care rapid immunoassay performed on voided urine, refrigerated up to 24 hours, accurately detects bacteriuria

David C. Grant, DVM, MS, DACVIM[1]*; Audrey E. Keebaugh, DVM, MS, DACVIM[1]; Stephen Werre, PhD[2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA
[2]Study Design and Statistics Laboratory, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

*Corresponding author: Dr. Grant (GrantInternalMedicine@gmail.com)

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OBJECTIVE
To collect voided urine from dogs with clinical signs of lower UTI and determine the diagnostic performance of a commercially available rapid immunoassay (RIA) immediately after urine collection and after refrigeration at 4 and 24 hours.

ANIMALS
40 client-owned dogs.

METHODS
Aerobic urine culture was performed on urine collected by cystocentesis. Urine samples were collected by voiding, and the RIA performed in triplicate within 30 minutes (time 0) and again in triplicate after 4 and 24 hours of refrigeration. Test precision and agreement between culture results and RIA results at each time point were determined, and factors possibly associated with false results investigated.

RESULTS
14 of 40 dogs (35%) had UTI verified by aerobic urine culture, and all had positive RIA. Three dogs had false positive RIA results. Sensitivity, specificity, positive predictive value, and negative predictive value of the RIA were 100, 88%, 82%, and 100%, respectively, and results were not different after 4 and 24 hours of refrigeration. Precision was excellent.

CLINICAL RELEVANCE
This point-of-care RIA, performed on voided urine refrigerated up to 24 hours, rapidly and accurately identifies bacteriuria in dogs with lower urinary tract clinical signs, inexpensively.

Keywords: rapid, urinary tract infection, voided, diagnosis, refrigerated

Aerobic culture of urine collected by cystocentesis is recommended by the International Society for Companion Animal Infectious Diseases for diagnosis in dogs suspected of having urinary tract infection (UTI).[1] The intent of these recommendations is to discriminate infection from noninfectious diseases that mimic UTI, and thereby promote antimicrobial stewardship and optimal patient care. However, awaiting urine culture results can delay treatment if a patient is infected as well as delay further diagnostics and treatment if a patient is not infected but is suspected to be.

There are benefits to urine collection by cystocentesis or by voided urine sample. Cystocentesis avoids potential contamination of urine samples by distal urethral commensal bacteria.[1,2] Collecting voided urine is simple, can be done at home or in the veterinary office, doesn't require restraint, doesn't induce pain, and doesn't cause potential iatrogenic complications. Cystocentesis has the potential to be dangerous in patients with clotting or coagulation disorders. Due to this simplicity of collecting voiding urine samples, dog owners collect voided urine at

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home from their dogs for analysis at their veterinarian's office, sometimes even under the instruction of their veterinarian to do so.

In recent years, there have been several publications reporting the use of a point-of-care, bacteriuria, rapid immunoassay (RIA) urine detection test (RapidBacVet; Silver Lake Research Corp).[3–5] The test takes approximately 20 minutes to perform, is much less expensive than urine culture, and studies suggest it is a useful screening test for bacteriuria in dogs. The test was used on 200 urine samples from dogs, but the study did not describe if dogs had lower urinary tract signs or were suspected of having a UTI. Using urine culture as the standard, it had sensitivity and specificity of 71.7% and 100%, respectively.[3] Ninety-two percent of the samples were collected by cystocentesis and 2% by voiding. Another study in 44 dogs with a suspicion of UTI, found the sensitivity and specificity to be 81.8% and 95.5%, respectively when using a definition of any bacterial growth as the standard, and 90.0% and 95.2%, respectively, when using a definition of $\geq 10^3$ CFU/mL.[4] To determine performance on urine collected by voiding, as this collection method can be more convenient to obtain for screening purposes, the RIA was performed on voided urine in 26 dogs with lower urinary tract signs, using culture of urine concurrently collected by cystocentesis as the standard.[5] The sensitivity and specificity was determined to be 89% and 100%, respectively. This study was underpowered and would benefit from a larger population.

Given the results of the aforementioned study, we wanted to further investigate the performance of the RIA on voided urine in a properly powered study.[5] Further, we wanted to determine the precision (repeatability) of the test as well as whether it maintained its diagnostic performance when urine samples had been refrigerated for up to 24 hours. We hypothesized, under these conditions, the positive and negative RIA results would correlate well with positive and negative bacterial cultures, at all time points, and the RIA tests run in triplicate would consistently determine the same result.

Methods

This prospective study was performed at the Virginia Maryland College of Veterinary Medicine from July 2021 to November 2022. Dogs were enrolled if they had at least 1 clinical sign of lower urinary tract disease, their veterinarian recommended aerobic bacterial culture of urine collected by cystocentesis, and their owner consented. Pollakiuria, stranguria, gross hematuria, periuria, excessive licking of the genital area, and urinary incontinence were considered lower urinary tract clinical signs.[5] There were no exclusion criteria. Urine spontaneously voided was collected into a clean ladle or plastic cup then transferred to sterile plastic or glass blood tubes. No attempt was made to clean the prepuce or vulva prior to collection nor to collect urine at a certain time during voiding. The RIA was performed per manufacturer instructions as outlined in a previous study on an aliquot of urine and the remainder placed in refrigeration for 24 hours.[5] The RIA was performed in triplicate within 30 minutes of collection (time 0) and again after 4 (time 4) and 24 hours (time 24) of storage at 40 °C. At 4 and 24 hours, the RIA was performed immediately after removing the urine sample from refrigeration; no attempt was made to warm the sample. The Gram classification feature of the RIA was not utilized for this study. Within ± 4 hours of collection of the voided sample, cystocentesis was performed followed by urinalysis and plating for aerobic bacterial culture within 30 minutes. Licensed veterinary technicians or veterinarians performed the RIA and were unaware of urinalysis results at Time 0 and urine culture results at Times 0, 4, and 24. Medical technologists of the VITALS lab of the Veterinary Teaching Hospital performed the urinalysis and aerobic urine culture and were unaware of the RIA test results at all time points.

Any bacterial growth on the culture plate was considered positive for bacteriuria and also for a diagnosis of UTI since the dogs had clinical signs consistent with UTI. If there were discrepancies in the 3 RIAs run at a time point, whichever result occurred twice was considered the correct diagnosis and used for comparison with urine culture. If performance of the RIA at certain times was not performed, the patient was excluded from statistical analysis at that time point. Five of the 120 examination time points in which the RIA was not able to be performed are shown **(Table 1)**.

Population size was determined a priori. Calculations were performed using data from prior studies.[3,5] We utilized UTI prevalence of 43% and 20%, 80% power, and *P* value .05.[3,5] From this, acquiring 30 to 60 dog urine samples (including at least 12 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. Sensitivity, specificity, and positive and negative predictive values for the RIA, using the urine culture result as the definitive result, and 95% CIs for each test time-point (times 0, 4, and 24) were calculated using standard equations. A χ^2 test (McNemar test) was performed to determine the association between the RIA and aerobic bacterial culture results at each time point. To assess agreement between bacterial culture and RIA results at each time-point and agreement between RIA triplicates, a simple kappa statistic (K) and 95% CIs were calculated. *P* values < .05 were considered significant.

Associations between RIA/urine culture discordant results (as an outcome) and baseline variables (1 variable at a time) were assessed using the Wilcoxon rank sum test (age) and Fisher's exact test (sex, pollakiuria, hematuria, stranguria, licking, periuria, and incontinence).

Results

Forty dogs were enrolled in the study. No dog was utilized twice. The average age was 6.3 years. There were 23 spayed-female, 7 female, 6 male, and 4 neutered-male dogs. Clinical signs included

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Table 1—RIA results (triplicate results) and urine culture results for all dogs.

Dog	Time 0 RIA			Time 4 RIA			Time 24 RIA			Urine culture (CFUs and species)
1	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	> 100K *Escherichia coli*
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	> 100K *E coli*
6	-	-	-	-	-	-	-	-	-	-
7	+	-*	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	-
9	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	-*	-	+	+	+	-
11	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	> 100K *E coli*
13	+	+	+	+	+	+	+	+	+	> 100K *E coli*
14	+	+	+	+	+	+	+	+	+	> 100K *Staphylococcus pseudintermedius* and *Enterococcus faecalis*
15	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	> 100K *E coli*
23	+	+	+	+	+	+	+	+	+	> 100K *Proteus mirabilis*
24	-	-	-	-	-	-	-	-	-	-
25	+	+	+	0	0	0	0	0	0	> 100K *E coli*
26	+	+	+	0	0	0	+	+	+	3,800 *S pseudintermedius*
27	+	+	+	0	0	0	+	+	+	-
28	+	+	+	+	+	+	+	+	+	> 100K *S pseudintermedius*
29	-	-	-	-	-	-	-	-	-	-
30	+	+	+	+	+	+	+	+	+	> 100K *P mirabilis*
31	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-
37	+	+	+	0	0	0	+	+	+	5,300 *E coli*
38	-	-	-	-	-	-	-	-	-	-
39	+	+	+	+	+	+	+	+	+	> 100K *E coli*
40	+	+	+	+	+	+	+	+	+	500 *E coli*

RIA = Rapid immunoassay.

*Indicates the RIA result that was utilized for statistical analysis when there was discrepancy in results of triplicate tests performed. +Indicates a positive test result. –Indicates a negative result. 0 indicates a test was not performed.

pollakiuria (24/40), periuria (15/40), incontinence (14/40), gross hematuria (11/40), stranguria (10/40), and excessive licking of genitalia (8/40), with 25 dogs having ≥ 2 signs. Urine cultures were determined to be positive in 14 dogs and negative in 26 dogs. One dog grew 100 cfu/mL of *Pseudomonas* species, which per the study design would be considered a positive urine culture. However, the veterinarian in charge suspected this was merely a contaminant, corrected the dog's concurrent disorder (ectopic ureters), and discharged the patient without antibiotics. The patient's clinical signs resolved within a day post-operatively and a subsequent urine culture was negative. Therefore, this dog was reclassified as having a negative urine culture. There were only 2 RIA triplicates with disagreement, 1 at time 0 and 1 at time 4 in separate dogs. In 1 dog (dog 7), at time 0, the majority RIA result was negative, which correlated with all RIAs at times 4 and 24 hours as well as with the urine culture result. In the other dog (dog 10), at time 4, the majority RIA result was negative, which was contrary to all RIAs at times 0 and 24 hours but consistent with the urine culture result. Thus, there was 1 discrepant RIA triplicate each at times 0 (2.5%) and 4 (2.5%) and none (0%) at time 24. The frequencies of discrepant results were not significantly different between time points (P = .607). Neither signalment nor any urinary tract signs were more common in dogs with false RIA results. Regarding precision within triplicates, there was strong agreement at times 0, 4, and 24 hours (k = 0.97 [CI, 0.79 to 1.1], k = 0.96 [0.79 to 1.1], and k = 1 [0.09 to 1.2]), respectively.

There were 14 dogs with positive urine cultures and all RIA tests from these dogs were positive at all time points measured with no false negative RIA results (Table 1). There were 26 dogs with negative urine cultures; 23 had negative RIA tests at all time

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Table 2—Diagnostic performance for the RIA, performed on voided urine, in diagnosing UTI in dogs with lower urinary tract signs.

	Time 0 RIA	Time 4 RIA	Time 24 RIA
Sensitivity (95% CI)	100% (78%–100%)	100% (74%–100%)	100% (77%–100%)
Specificity (95% CI)	88% (71%–96%)	96% (8%–99%)	88% (71%–96%)
PPV (95% CI)	82% (59%–94%)	92% (65%–99%)	81% (57%–0.93%)
NPV (95% CI)	100% (86%–100%)	100% (86%–100%)	100% (86%–1%)

NPV = Negative predictive value. PPV = Positive predictive value. Time 0 = Immediately after urine collection. Time 4 = After 4 hours of refrigeration. Time 24 = After 24 hours of refrigeration.
See Table 1 for remainder of key.

points measured, 2 had positive RIA results at all time points measured, and 1 had positive RIA results at times 0 and 24, but discrepant results at time 0 with the majority (2 of 3) RIA replicates being negative.

The sensitivity, specificity, and positive and negative predictive values of the RIA are shown **(Table 2)**. Results of urine culture and RIA were not independent at times 0, 4, and 24 (P = .08, P = .31, P = .08). There was strong agreement between urine culture results and RIA results at times 0, 4, and 24 (k = 0.84 [CI, 0.67 to 1.0], k = 0.9 [0.81 to 1.0], k = 0.84 [0.66 to 1.0]).

Discussion

We investigated the performance of an RIA for detection of bacteriuria in dogs with lower urinary tract signs by using voided urine with the hypothesis the test would correlate well with the gold-standard, culture of urine collected by cystocentesis. The diagnostic performance of the RIA, performed immediately after urine collection (time 0), in previous and current studies are very similar: sensitivity, 89% versus 100%; specificity, 100% versus 88%; positive predictive value, 100% versus 82%; and negative predictive value (NPV), 92% versus 100%.[5] The excellent diagnostic performance was no different when the same voided urine was stored in refrigeration for 4 and 24 hours. This diagnostic performance is similar to a previous report (sensitivity, 81.8% and specificity, 95.5%) when the RIA was performed on urine collected by cystocentesis.[4]

This RIA, performed on voided urine and according to the product instructions, appears to be an excellent test to screen dogs, with lower urinary tract signs, for UTI. As there were no false negative results, a negative result could allow more expeditious diagnostic pursuit of the true cause of clinical signs, rather than waiting days to a week for culture results and avoidance of unnecessary antibiotic prescription. A positive diagnosis would ideally be followed by submission of a urine sample for aerobic culture to confirm the diagnosis of UTI because of the low percentage of false positives, but more importantly to obtain antibiotic susceptibility results and to confirm or deny the diagnosis of UTI. The RIA can be completed during a patient outpatient visit, making this screening test practical and efficient. Diagnosis of bacteriuria by urinalysis can be complicated by numerous factors. Urinalysis performed by experienced medical technologists in a college of veterinary medicine on unstained urine sediment has sensitivity, specificity, and positive and negative predictive values of 82.4%, 76.4%, 40.1%, and 95.8%, respectively when compared to culture of urine by cystocentesis.[6] This performance is lesser than the current RIA. When a modified Wright stain is applied to air dried urine sediment slides and interpreted by a single board-certified clinical pathologist, the reported sensitivity, specificity, and positive and negative predictive values were 93.2%, 99.0%, 94.5%, and 98.7%, respectively.[6] While these compare more favorably than the diagnostic values for the RIA, it seems unlikely the average veterinary staff would be as accurate in this task and unlikely to submit urine to a clinical pathologist for this purpose. The RIA does not require a veterinarian to perform it. In a prior study, all RIAs were performed by licensed veterinary technician as were most in the current study.[5] A recent report using an automated analyzer to identify bacteriuria in cats reported sensitivity of 100% but a very low specificity of 35%.[7] This low specificity would lead to many unnecessary urine cultures being recommended. Another recent study evaluating the performance of 2 automated urine analyzers for identifying bacteria in dogs and cats reported sensitivity, specificity, and positive and negative predictive values of 82.9% and 89.8%, 81.1% and 72.3%, 54.1% and 47.3%, and 94.6 and 96.2%, respectively.[8] These analyzers have many false positive results, again likely leading to a large number of unnecessary urine cultures being recommended.

The RIA was also precise, with discrepancies within triplicates occurring rarely (2/120) and no more often after storage than immediately after urine collection. This is the first study to evaluate precision and the effects of storage on the RIA in dogs. Further, the discrepant results were not associated with any patient or clinical sign variables examined (sex, age, pollakiuria, gross hematuria, stranguria, excessively licking genitalia, periuria, or incontinence). Thus, it appears it may be practical for a dog-owner to collect urine at home, and, if necessary, store the sample in their refrigerator, optimally in a sterile container, until transport at 40F to their veterinarian's office within 24 hours for the RIA to be performed. This has the potential to increase the number of dogs who can successfully have urine collected at the time of clinical signs, reduce dog transport and resultant anxiety, and avoid a veterinarians need to accommodate an urgent patient and client need for an office visit.

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The gold-standard for diagnosis of UTI, according to ISCAID, is culture of urine collected by cystocentesis in dogs with clinical signs of UTI.[1] Thus, if only a urine sample had been brought to a veterinarian without the dog and a positive RIA occurred, the dog would, ideally, be brought to the veterinarian for cystocentesis. This may then result in a second travel event for the dog owner. At the same time, in our study, with a 100% NPV and most dogs having a negative RIA result, owners of such dogs would avoid a much more costly urine culture, and diagnostics and treatments to address the correct diagnosis would proceed more quickly. The 100% NPV makes it clear which dogs should undergo cystocentesis, a procedure which could cause some degree of pain, can cause anxiety, and carries a degree of risk of adverse effects.

There were a small number (5/120) of missing RIA observations at times 4 and 24. These occurred because staff were either too busy to perform the RIA or were asleep when it was to be performed. It seems unlikely a 4% lack of data would alter results. The number of dogs (40) may be considered small, but when put in context of very similar results in prior studies of this RIA it is likely the results of this study accurately reflect what would be found in the general dog population.[3–5] Sutter et al[4] found the sensitivity of the RIA on samples with bacterial colony counts < 103 (2 samples) to be 0% whereas for samples > 103 the sensitivity was 90% (all on urine collected by cystocentesis).[4] This suggests the RIA may not detect low colony counts. Our study did not include any counts this low, thus the efficacy of the RIA on such samples remains uncertain.

In conclusion, this simple point-of-care RIA test can be performed in-office, rapidly, at low-cost, and without specialized training. Results are reliable even when urine samples are collected by voiding and refrigerated for up to 24 hours. Using this RIA and collection method may lead to monetary savings, facilitate more rapid patient diagnosis and treatment, and (when performed on dogs with lower urinary tract clinical signs) clarifies which dogs should have urine culture and susceptibility performed.

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Disclosures

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