Objective
To determine the diagnostic accuracy of a rapid immunoassay (RIA) for point-of-care detection of urinary tract infection (UTI) of dogs, compared with criterion-referenced diagnosis with bacterial culture.

Sample
200 urine samples obtained from dogs and submitted to a veterinary microbiology diagnostic laboratory for routine bacterial culture and antimicrobial susceptibility determination.

Procedures
Samples were evaluated by use of quantitative bacterial culture and the RIA. Sensitivity, specificity, and positive and negative predictive values of the RIA were calculated; results of bacterial culture were the criterion-referenced outcome. A κ statistic was calculated to determine agreement between bacterial culture and RIA results.

Results
56 of 200 (28%) urine samples had positive results for bacterial growth by use of culture methods; there were 38 (19%) positive results likely to be associated with bacterial UTI on the basis of sample collection method and bacterial concentration. Sensitivity and specificity of the RIA for detecting samples likely to be associated with UTI (≥1,000 CFUs/mL) were 97.4% and 98.8%, respectively. The positive and negative predictive values of the RIA were 0.949 and 0.994, respectively. Agreement between bacterial culture and RIA outcome for UTI was substantial (weighted κ, 0.718).

Conclusions and Clinical Relevance
The RIA test evaluated in this study accurately detected UTI of dogs, compared with detection with the criterion-referenced bacterial culture method. Use of this point-of-care RIA could allow clinicians to diagnose UTI at the time of a patient visit and provide information useful for immediately initiating empirical antimicrobial treatment. (Am J Vet Res 2016;77:162–166)
examination was found to improve sensitivity and specificity, compared with results for urine sedimentation examination (urinalysis) alone. However, this approach may require specialized materials, equipment, expertise, and time that are not routinely available in primary care veterinary clinics. In addition, staining techniques require a critical threshold of organisms for detection, unless a centrifugation step is included. A rapid catalase-based test has been used to detect UTI of dogs and cats, but this test lacks sensitivity and specificity and requires bacterial culture to confirm UTI of dogs.

The objective of the study reported here was to evaluate a newly marketed RIA for use in detecting UTI of dogs. If accurate, this RIA may offer a point-of-care diagnostic test for UTI that is self-contained and easily implemented at veterinary clinics without the need for specialized equipment or expertise. In addition, the RIA would provide information on the type of bacteria likely to be present, which should lead to improvements in the choice of empirical treatment.

Materials and Methods

Samples

Canine urine samples (n = 200) submitted to the North Carolina State Veterinary Hospital Clinical Microbiology Laboratory for routine aerobic culture were used in the study. Samples were collected between April and September 2014 via cystocentesis, with a urinary catheter, or during urine voiding. Samples were placed in a transport system and submitted for testing.

Experimental procedures

An aliquot (300 µL) of each eligible urine sample (determined on the basis of an adequate sample volume) was removed and evaluated in duplicate for bacteriuria by use of an RIA kit. The remainder of each urine sample was subjected to standard laboratory methods for bacterial identification and quantification. Samples were enrolled on the basis of convenience for laboratory personnel; there were no exclusion criteria. Most samples were processed within 2 hours after receipt at the laboratory; however, all samples were processed on the same day they were collected (within the time frame and conditions for the transport system).

The RIA was performed in accordance with manufacturer instructions. Briefly, 150 µL of assay diluent was placed into vials for each sample and a replicate of each sample. The diluent was incubated at room temperature (22°C) for 2 minutes; samples were stirred during incubation to dissolve the conjugate. Urine (150 µL) was added to each vial, and vials were incubated (with occasional stirring) for 5 minutes at room temperature. After incubation was complete, an RIA test strip was placed into each sample vial. The strip was allowed to remain in place for 10 minutes. It was then removed, and results were interpreted in accordance with manufacturer instructions. All results were recorded, and all test strips were photographed.

Each test strip had a control band and 2 additional bands that might be visible, depending on the type of bacteria present in a urine sample. The presence of band 1 (with or without the presence of band 2) indicated UTI caused by gram-negative bacteria (including Escherichia coli, Klebsiella spp, Citrobacter spp, Enterobacter spp, and Serratia spp), and the presence of band 2 alone indicated UTI caused by gram-indeterminate bacteria (including Proteus spp, Pseudomonas spp, Morganella spp, Providencia spp, Staphylococcus spp, Enterococcus spp, Actinomyces spp, and Actinobaculum spp). All bands were scored in accordance with a scale provided by the manufacturer. Scores were assigned on the basis of the intensity of color by use of a scale from 0 to 5 (0 = no band was evident [negative result], 1 = band was extremely light and difficult to see, 2 = band was light but easily visible, 3 = band was of moderate intensity, 4 = band was dark, and 5 = band was extremely dark and almost black). One investigator (MDC) was responsible for performing all RIA testing and interpreting all RIA results.

The remainder of each urine sample was processed in accordance with laboratory standard operating procedures. Briefly, a 10-µL calibrated loop was used to streak each urine sample onto 5% Columbia blood agar and MacConkey agar; these plates were incubated in an environment with 5% CO₂ or ambient air, respectively, at 36°C for 18 to 24 hours. The remainder of each sample was inoculated into 20 mL of thioglycollate medium for enrichment. These tubes were incubated in an environment with ambient air at 36°C for 18 to 24 hours.

After incubation was complete, plates with bacterial growth were quantified and evaluated for identification by use of biochemical testing and Gram staining. Samples with bacterial growth from the enrichment-only medium were quantified as < 1,000 CFUs/mL and, similar to the other bacteria, characterized by use of biochemical testing and Gram staining. If no growth was observed after the enrichment broth was plated, the sample was considered negative.

Patient identification, density of the bands, collection method, and culture outcome were recorded for each sample. Culture outcome was categorized in 2 ways. One categorization was culture-positive or -negative for any bacterial growth. The other categorization was whether the culture result was or was not likely to be associated with UTI; samples that were collected via cystocentesis and yielded a final bacterial concentration ≥ 1,000 CFUs/mL were considered likely to be associated with UTI.

Data analysis

Sensitivity, specificity, and positive and negative predictive values of the RIA were considered for both categorizations of culture outcome. In addition, a χ² test was performed to assess the association
between culture and RIA outcomes. A receiver operating characteristic curve was generated with sensitivity and specificity of the RIA (only for samples likely to be associated with UTI). A weighted κ statistic and 95% confidence interval were calculated to assess agreement between bacterial culture outcome (negative results, gram-positive bacteria, or gram-negative bacteria) and RIA outcome (negative results, gram-indeterminate bacteria, or gram-negative bacteria). Interpretation of the κ statistic was based on an accepted scale. A Cochran-Mantel-Haenszel statistic was calculated to test for the correlation between bacterial concentration (<1,000 CFUs/mL, 1,000 to 100,000 CFUs/mL, and >100,000 CFUs/mL) and band intensity. Values were considered significant at \( P < 0.05 \).

**Results**

Of the 200 canine urine samples, 184 (92%) were collected via cystocentesis, 11 (5.5%) by use of a urinary catheter, and 4 (2%) during voiding; the collection method for 1 urine sample was not known. Demographics of dogs were not collected.

Fifty-six (28%) urine samples had positive results for any bacterial growth by use of a criterion-referenced method (bacterial culture). These included samples with multiple bacterial species. The number of samples likely to be associated with bacterial UTI, as determined on the basis of quantitative bacterial culture and method of sample collection, was 38 (19%). Of the 56 cultures with any bacterial growth, 35 (62.5%) had >10,000 CFUs/mL, and 1 culture each had 5,000 to 10,000 CFUs/mL, 60,000 to 70,000 CFUs/mL, and 70,000 to 80,000 CFUs/mL, respectively. Eighteen of 56 (32%) samples with bacterial growth yielded <1,000 CFUs/mL; these were considered unlikely to be associated with UTI on the basis of the study criteria. Three of 4 urine samples obtained during voiding yielded <1,000 CFUs/mL; 1 of these samples comprised 2 types of bacteria. The sample obtained by use of an unknown collection method yielded no bacterial growth. Three urine samples (regardless of collection method) had >1 possible pathogen. The most common isolates included *Escherichia coli* (n = 31), *Proteus mirabilis* (7), and *Enterococcus faecalis* (6; Table 1).

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of isolates</th>
<th>No. of urine samples in which organism was cultured with &gt;1 other bacterial species</th>
<th>No. of urine samples for which culture yielded &lt;1,000 CFUs/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>31</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus spp</em></td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><em>Corynebacterium spp</em></td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
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The RIA results for duplicate urine samples were identical; therefore, results were described for each urine sample submitted. Sensitivity and specificity of the RIA for detecting any bacterial growth in urine samples was 71.7% and 100%, respectively. Positive predictive value of the RIA for detecting any bacterial growth was 1.000, whereas the negative predictive value was 0.906. Sensitivity of the RIA for detecting urine samples likely to be associated with UTI (collected via cystocentesis and yielded ≥1,000 CFUs/mL) was 97.4%, whereas specificity was 98.8%. Positive and negative predictive values of the assay for urine samples likely to be associated with UTI (collected via cystocentesis and yielded ≥1,000 CFUs/mL) were 0.949 and 0.994, respectively. The RIA had false-positive results for 2 urine samples that yielded <1,000 CFUs/mL (and therefore were unlikely to be associated with UTI); those 2 samples contained *E. coli* and *Klebsiella pneumoniae*, respectively. The RIA had a false-negative result for 1 urine sample that yielded 5,000 CFUs/mL.
to 10,000 CFUs/mL (E. coli and P. mirabilis) and was considered likely to be associated with UTI. The area under the receiver operating characteristic curve was 0.848 (Figure 1).

Agreement between culture outcomes (negative, gram-positive, or gram-negative bacterial identification) for samples likely to be associated with UTI and RIA outcomes (negative, gram indeterminate, or gram negative) was substantial; the weighted κ was 0.718 (95% confidence interval, 0.61 to 0.83). One sample that yielded > 100,000 CFUs/mL (contained both E. coli and E. faecalis) had gram-negative results for the RIA; all other samples with multiple organisms on bacterial culture were appropriately categorized by use of the RIA.

The control band of the RIA was apparent for all samples. Mean and median intensity of the control band was 3.6 and 4.0, respectively. Mean intensity of band 1 and band 2 for RIA tests with positive results was 2.3 and 3.4, respectively. For both band 1 and band 2, there was a significant (P < 0.001) correlation between band intensity and bacterial concentration for culture.

**Discussion**

In the study reported here, canine urine samples submitted to a veterinary diagnostic microbiology laboratory for routine testing were used to determine the diagnostic accuracy of a newly marketed RIA for detecting UTI in dogs. The prevalence of likely bacterial UTI in dogs of this study (38/200 [19.0%]), as determined on the basis of quantitative bacterial culture and method of sample collection, was within the range reported for other diagnostic evaluation studies.6,7,9,13 This was worthy of mention, especially considering the referral nature of the samples enrolled and the influence of prevalence for some outcomes of diagnostic accuracy. We also determined accuracy of the RIA for detection of urine samples yielding any bacterial growth in the event a urine sample was collected by a method other than cystocentesis or the sample yielded < 1,000 CFUs/mL but was considered to be associated with bacterial UTI. The bacterial species recovered in the present study were primarily represented by E. coli strains and were similar to those in previous reports13,14 of bacterial UTI in dogs.

Other diagnostic tests evaluated for detection of UTI in dogs failed to provide results in a timely manner, were not available as point-of-care tests, required specialized materials or expertise, or lacked appropriate sensitivity and specificity to be used routinely.5-9 In the study reported here, only samples that had ≥ 1,000 CFUs/mL and were collected via cystocentesis were considered likely to be associated with bacterial UTI. The RIA evaluated in the present study had high sensitivity (97.4%) and specificity (98.8%) for detecting bacterial UTI, compared with UTI diagnosed on the basis of quantitative bacterial culture and method of sample collection. The positive and negative predictive values, which are a measurement of the dogs with or without UTI and correctly identified by the RIA, were also strong (0.949 and 0.994, respectively). The RIA failed to identify (false-negative results) 1 sample that met the criteria; that sample contained 5,000 to 10,000 CFUs/mL and contained both P. mirabilis and E. coli. The RIA detected all other urine samples of dogs with UTI. Additionally, the RIA detected 2 samples containing < 1,000 CFUs/mL, which were collected from dogs unlikely to have bacterial UTI (false-positive results). All other quantitative bacterial culture samples that yielded no bacteria or low numbers of bacteria had negative results when tested with the RIA.

The RIA evaluated in the study reported here was a self-contained kit that required no specialized equipment. Results were available approximately 20 minutes after start of the RIA. Outcome was determined as the presence or absence of a control band and 1 or 2 bacterial-detection bands on each test strip. Intensity of bands on the test strip was strongly correlated with bacterial concentration in the urine sample. All samples were assayed in duplicate; however, results (including intensity of bands for test strips with positive results) were identical for both duplicates.

It was beyond the scope of the present study to determine specific antimicrobial susceptibility profiles for the obtained isolates. Antimicrobial use as a result of suspected or confirmed bacterial UTI in dogs is common.6 Antimicrobial resistance has been reported for bacterial isolates obtained from the canine urinary tract.13,14 Because of increasing concerns about antimicrobial resistance, including the potential for increased morbidity and mortality rates, judicious antimicrobial use has been addressed by veterinary medicine task forces and policies.15,16 Recommendations in these policies frequently include the use of culture and susceptibility testing for accurate diagnosis and treatment.15 However, it remains common practice to initiate antimicrobial treatment to dogs suspected of uncomplicated UTI before such results are available.

The RIA described in the present study did not include information on antimicrobial susceptibility. Categorization of samples with positive results as gram negative or gram indeterminate may assist a veterinarian in selection of an appropriate empirical treatment for a particular pathogen or group of pathogens. This would likely be more applicable for tests identifying gram-negative bacteria because the gram-indeterminate category contained both gram-positive and gram-negative organisms. Although this study was not designed to determine RIA accuracy for various bacterial species, this information would also be of interest and useful when making prudent decisions regarding antimicrobial treatment.

In addition, a point-of-care test that provides immediate results, including those indicating that a dog is not likely to have UTI, may reduce the number of treatments prescribed without diagnostic testing and prevent delays in performing other potentially useful diagnostic tests while awaiting culture results. This RIA is not likely to replace the need for bacterial culture and antimicrobial susceptibility testing in all
cases, particularly for dogs with complicated or recurrent UTI. However, results would be rapidly available, which may lead to improved information at the initial patient evaluation. It would be of interest to determine the number of bacterial-associated UTIs identified by the RIA that would fail to respond to empirical treatment.

In the present study, a recently marketed RIA was evaluated to determine its diagnostic accuracy for detecting UTI in dogs, compared with diagnosis based on quantitative bacterial culture. The RIA test accurately detected UTI, compared with results for the criterion-referenced bacterial culture. This RIA may provide a reliable point-of-care option for clinicians attempting to diagnose UTI during an office visit. Although not a substitute for bacterial culture and susceptibility testing, the RIA can provide generalized information on the organisms contributing to UTI, particularly when they are gram-negative bacteria, which may improve empirical antimicrobial treatment.

Acknowledgments

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Footnotes

a. Port-a-cul, BD, Franklin Lakes, NJ.
b. RapidBac Vet, Silver Lake Research Corp, Monrovia, Calif.
d. BBL, BD, Franklin Lakes, NJ.
e. Sensititre, Trek Diagnostic Systems, Thermo-Fisher, Oakwood Village, Ohio.

References