Urinary tract infections (UTIs) are frequently encountered in clinical practice and most commonly caused by *Escherichia coli* and other Gram-negative uropathogens. We tested RapidBac, a rapid immunoassay for bacteriuria developed by Silver Lake Research Corporation (SLRC), compared with standard bacterial culture using 966 clean-catch urine specimens submitted to a clinical microbiology laboratory in an urban academic medical center. RapidBac was performed in accordance with instructions, providing a positive or negative result in 20 min. RapidBac identified as positive 245/285 (sensitivity 86%) samples with significant bacteriuria, defined as the presence of a Gram-negative uropathogen or *Staphylococcus saprophyticus* at ≥10^5 CFU/ml. The sensitivities for Gram-negative bacteriuria at ≥10^4 CFU/ml and ≥10^5 CFU/ml were 96% and 99%, respectively. The specificity of the test, detecting the absence of significant bacteriuria, was 94%. The sensitivity and specificity of RapidBac were similar on samples from inpatient and outpatient settings, from male and female patients, and across age groups from 18 to 89 years old, although specificity was higher in men (100%) compared with that in women (92%). The RapidBac test for bacteriuria may be effective as an aid in the point-of-care diagnosis of UTIs especially in emergency and primary care settings.

Bacterial urinary tract infections (UTIs) are a common clinical problem across the age spectrum in both genders (1). Women and girls are disproportionately affected by UTIs, with the lifetime risk estimated at >60% (2). In the United States, the overall annual cost of diagnosis and treatment of UTIs is considerable, estimated at $2.3 billion in 2010 (1). Gram-negative bacteria are the causative agents in up to 95% of uncomplicated UTIs in women, with *Escherichia coli* responsible for 70% to 90% (1). Diagnosis of UTI remains problematic in certain settings. The presence of uropathogenic bacteria in urine is the hallmark of UTI, and urine culture is the gold standard method for determination of clinically relevant bacteriuria. However, the 24- to 48-h delay in obtaining urine culture results has presented a longstanding need for more rapid diagnostic methods. Currently available rapid methods for detection of bacteriuria, including microscopy and test strips for detecting nitrite, have been shown to have poor sensitivity (3–5). A meta-analysis of 34 studies evaluating the accuracy of nitrite test strips across settings and populations found a mean sensitivity of 48% (using a definition of 10^5 CFU/ml for significant bacteriuria) (6). Microscopic urinalysis and urine Gram staining, two relatively laborious methods, have been shown by several studies to lack sensitivity below 10^4 CFU/ml and to have poor specificity (4, 5, 7, 8). Guidelines for UTI from the Infectious Disease Society of America (IDSA) do not recommend urine culture for most cases of acute uncomplicated cystitis, the most common UTI presentation, and do not address laboratory methods for diagnosing UTI (9).

An accurate and rapid point-of-care diagnostic method for detection of bacteriuria would be a powerful new aid in the diagnosis and appropriate treatment of UTIs and in the differential diagnosis of UTI versus that of other pathologies presenting with similar symptoms. We report here the results of an evaluation of a new rapid test kit, RapidBac, a direct lateral flow immunoassay for bacteria in urine. The goal of the present study was to determine the sensitivity and specificity of RapidBac in the detection of bacteriuria, using urine culture as the gold standard method. The results of this study may provide a rationale for implementation of rapid point-of-care diagnostics in the diagnosis and treatment of suspected UTIs in a variety of clinical settings.

**MATERIALS AND METHODS**

**Study design.** Under an institutional review board (IRB)-approved protocol, personnel in the University of Washington Medical Center (UWMC) Clinical Microbiology Laboratory prepared standardized aliquots in a boric acid-based preservative of deidentified, to-be-discarded, clean-catch urine samples (n = 966) collected from adults aged 18 to 89 attending UW Medicine outpatient clinics or hospital and submitted with orders for urine culture. The specimens were assigned study numbers and an honest broker (10) was used to create a log correlating study numbers with clinical laboratory numbers. The aliquots were then tested using the Silver Lake Research Corporation (SLRC) RapidBac test kit. The results of RapidBac were then compared with urine culture results from the UWMC Clinical Microbiology Laboratory. The honest broker collected basic demographic data, including age, gender, inpatient or outpatient status, and date of collection.

**Bacteriuria test kits.** The RapidBac test is a lateral flow immunoassay test strip, similar to a home pregnancy test, in which monoclonal antibodies specific for bacterial cell surface determinants bind the target bacteria in a “sandwich” manner, producing a visual signal when bacteria are de-
in the samples. An indeterminate result was defined as a test result that could not be definitively classified as positive or negative for significant bacteriuria due to the presence of a Gram-negative organism. It is presumed that the RapidBac test was positive due to the presence of the Gram-negative organism.

Clinical Microbiology Laboratory urine culture protocols. Urine culture and pathogen identification were performed at the UWMC Clinical Microbiology Laboratory by previously described methods (12, 13). For clean-catch urine specimens, routine species-level identification is performed only on organisms in quantities of ≥10<sup>5</sup> CFU/ml, where no more than 2 organisms are present at this quantity (14). The laboratory report includes total colony count, semiquantitation (1+ to 4+), and organism identification when deemed appropriate or presumptive characterization when growth does not meet the criteria for species-level identification (e.g., mixed Gram-positive flora). Semiquantitative results were then converted to corresponding colony counts.

Urine culture results were classified as follows. Samples were classified as having significant bacteriuria if the laboratory identified ≥10<sup>5</sup> CFU/ml of a Gram-negative uropathogen or Staphylococcus saprophyticus. Samples with no growth or reported as mixed Gram-positive flora were classified as no significant bacteriuria. All other samples were classified as indeterminate, including those for which (i) potential pathogens were present at <10<sup>5</sup> CFU/ml, (ii) the presence of >2 organisms of equal quantity yielded no species-level identification of any Gram-negative bacteria, or (iii) any single Gram-positive organism that was not S. saprophyticus was isolated.

Because the study was designed to utilize deidentified, discarded samples with limited clinical information, the indeterminate group included samples for which interpretation of the laboratory report would require clinical correlation to determine clinical relevance of the detected bacterium, such as group B streptococci (15). These samples were not used for sensitivity and specificity determination since the urine culture result could not be classified as showing or not showing significant bacteriuria.

Statistical methods. Data were summarized into 2-way tables using SAS software, and sensitivity and specificity measures were constructed by applying the standard formulas to the data. Evaluation of the RapidBac sensitivity was performed using samples classified as significant bacteriuria. Evaluation of RapidBac test kit specificity was performed using samples classified as “no significant bacteriuria.” Indeterminate samples were excluded from the analysis of sensitivity and specificity.

Discrepant analysis. Specimens testing positive with the RapidBac test kit but negative for significant bacteriuria (n = 31) were further investigated to determine whether an identifiable organism was detectable and potentially the cause of the positive result. All urine samples tested with the RapidBac kit were independently cultured in parallel in one of the author’s independent research laboratories (A.E.S.). For all organisms recovered on the original culture plate, each colony type was isolated and retested individually with the RapidBac kit to determine which organism was associated with the positive result, then identified by the UWMC Clinical Microbiology Laboratory using standard microbiological methods or genetic sequencing (16). For one urine sample yielding no bacteria on the original culture plate and one in which no organism present on the original plate accounted for RapidBac positivity, the urine samples were centrifuged and the pellets were Gram stained, plated on a panel of standard and selective clinical microbiological media to detect typical uropathogens as well as more fastidious organisms, and incubated for 3 days in aerobic and anaerobic conditions and in 5% CO₂.

RESULTS

Sensitivity and specificity of the RapidBac bacteriuria detection kit. Urine culture results (n = 966) from evaluative, clean-catch urine samples were designated significant bacteriuria (n = 285, 30%), no significant bacteriuria (n = 540, 56%), or indeterminate (n = 141, 15%). The sensitivity of the RapidBac kit for any significant bacteriuria was 86% and 90% for significant bacteriuria with Gram-negative uropathogens (Table 1). The specificity of the RapidBac test was also high at 94% (Table 1). Among the 285 samples with significant bacteriuria, S. saprophyticus was cultured from 16 (6%, Table 2). Of these, 2 samples tested positive with the RapidBac kit due to the presence of Gram-negative bacteria in the samples.

Correlation of colony counts with RapidBac kit results. The sensitivity of the RapidBac kit for Gram-negative uropathogens was correlated with the colony count of uropathogens in the urine cultures (Table 3). Most samples identified by culture to have significant Gram-negative bacteriuria had ≥10<sup>5</sup> CFU/ml of the
specific uropathogen (151/270, 56%), and the RapidBac kit was positive for 150 of these (sensitivity = 99%). The sensitivity of the RapidBac kit on samples with significant Gram-negative bacteriuria below 10^5 CFU/ml was 79%.

**Sensitivity of the SLRC test kit for specific uropathogens.** Table 2 lists the percentage of samples with individual species of Gram-negative and Gram-positive uropathogens detected by the RapidBac kit in the significant bacteriuria sample population.

**RapidBac results in demographic subgroups.** The performance of RapidBac was analyzed in demographic subgroups (Table 4). The sensitivities observed between samples collected from male and female subjects or between inpatient and outpatient samples were similar. Specificity of the RapidBac kit on samples collected from men was 100% compared with 92% specificity for samples collected from women. The sensitivity and specificity of RapidBac did not change with age, except the specificity for samples collected from women. The sensitivity of the RapidBac kit on samples from subjects over the age of 30 was 90%.

**Analysis of discrepancies.** Among the 31 urine samples with no significant bacteria but positive by RapidBac, in most cases, a Gram-positive organism not usually associated with UTI appeared responsible for the discrepant result, e.g., Corynebacterium spp., Gardnerella vaginalis, or Actinomyces spp. Nonrecoverable Gram-negative rods were detected by urine Gram staining in 1 sample (3%). In 3 samples (10%), E. coli was detected at 10^6 CFU/ml in the research lab. Four samples (13%) yielded Gram-positive organisms not generally recognized as uropathogens but occasionally associated with UTI in case reports: Actinobaculum schaalii (17–20), Actinomyces europeaus (21, 22), Actinomyces urogenitalis (23), and Actinomyces neuiii (24). In summary, detailed studies in the research laboratory showed that in 8/31 (26%) samples positive by RapidBac but without significant bacteriuria, there was evidence of low-count Gram-negative bacteriuria or of a potentially uropathogenic Gram-positive organism, albeit in amounts not quantifiable using these methods.

The patient record was interrogated for the 19 samples that remained discrepant with no evidence of bacteriuria found in the research laboratory. Two samples were test-of-cure samples from individuals with recent UTIs; the presence of noncultivable and possibly nonviable Gram-negative bacteria in these samples would not be unexpected. Four samples came from patients with one or more symptoms of UTI.

Forty samples showed significant bacteriuria by culture but were negative with the RapidBac kit. Of these samples, 14 grew S. saprophyticus and 14 grew low concentrations of Gram-negative uropathogens (<10^4 CFU/ml).

**Results in the indeterminate group.** The results of the RapidBac kit on samples with indeterminate bacteriuria by culture were also compared to culture results (Table 5). Samples with low colony counts of uropathogens (<10^3 CFU/ml) and samples with Gram-positive organisms had low rates of positive results with the SLRC test kit (4/31, 13%). Samples with Gram-negative organisms, which the UWMC Clinical Microbiology Laboratory did not identify because the culture result did not meet the criteria required to proceed with identification at the species level, had a 35% positive result rate by RapidBac.

### TABLE 3 Sensitivity of RapidBac at different uropathogen concentration levels and thresholds

<table>
<thead>
<tr>
<th>Colony count* (CFU/ml)</th>
<th>No. of RapidBac-positive samples (sensitivity [%])</th>
<th>Cumulative no. of RapidBac-positive samples (sensitivity [%])</th>
<th>Cumulative total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^3</td>
<td>12 (46)</td>
<td>26</td>
<td>244 (90)</td>
</tr>
<tr>
<td>10^2</td>
<td>82 (88)</td>
<td>93</td>
<td>232 (96)</td>
</tr>
<tr>
<td>10^1</td>
<td>150 (99)</td>
<td>151</td>
<td>150 (99)</td>
</tr>
</tbody>
</table>

* Colony count categories encompass all samples at this log level.

### TABLE 4 Sensitivity and specificity of RapidBac by gender, sample source, and age

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total no. of samples</th>
<th>No. of RapidBac-positive samples/total no.</th>
<th>Sensitivity (%)</th>
<th>No. of RapidBac-negative samples/total no.</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>766</td>
<td>22/25</td>
<td>88</td>
<td>157/157</td>
<td>100</td>
</tr>
<tr>
<td>Male</td>
<td>200</td>
<td>244/260</td>
<td>86</td>
<td>352/383</td>
<td>92</td>
</tr>
<tr>
<td><strong>Sample Source</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>205</td>
<td>21/25</td>
<td>84</td>
<td>144/151</td>
<td>95</td>
</tr>
<tr>
<td>Outpatient</td>
<td>761</td>
<td>244/260</td>
<td>86</td>
<td>356/389</td>
<td>94</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–30</td>
<td>234</td>
<td>57/77</td>
<td>74</td>
<td>117/130</td>
<td>90</td>
</tr>
<tr>
<td>31–40</td>
<td>167</td>
<td>35/40</td>
<td>88</td>
<td>100/107</td>
<td>94</td>
</tr>
<tr>
<td>41–50</td>
<td>129</td>
<td>28/31</td>
<td>90</td>
<td>68/71</td>
<td>96</td>
</tr>
<tr>
<td>51–60</td>
<td>142</td>
<td>38/41</td>
<td>93</td>
<td>76/76</td>
<td>100</td>
</tr>
<tr>
<td>61–70</td>
<td>146</td>
<td>44/50</td>
<td>88</td>
<td>72/74</td>
<td>97</td>
</tr>
<tr>
<td>71–88</td>
<td>148</td>
<td>43/46</td>
<td>94</td>
<td>76/82</td>
<td>93</td>
</tr>
</tbody>
</table>
TABLE 5 Results of RapidBac on samples in the indeterminate group

<table>
<thead>
<tr>
<th>Culture result</th>
<th>No. of samples</th>
<th>No. of RapidBac-positive samples</th>
<th>% RapidBac-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uropathogen at &lt;10⁵ CFU/ml</td>
<td>8</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Gram-negative bacteria, not identified at the species level</td>
<td>109</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Gram-positive bacteria, not <em>S. saprophyticus</em></td>
<td>23</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Unable to quantitate</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>42</td>
<td>30</td>
</tr>
</tbody>
</table>

DISCUSSION

Rapid and convenient point-of-care methods for diagnosis of UTI are needed, especially in emergency and primary care settings. Although current guidelines for treatment of uncomplicated UTI in premenopausal women advocate empirical treatment based on *E. coli* as the predominant uropathogen, even among women of this demographic, urine culture is sometimes necessary. Examples of patients in whom accurate point-of-care diagnostics would be useful include those with atypical or subtle UTI symptoms, symptoms suggestive of sexually transmitted infections (STI), and patients unable to report symptoms. We evaluated the accuracy of a new rapid point-of-care diagnostic test kit for the determination of bacteriuria, using urine culture as the gold standard and testing urine samples from inpatient and outpatient adults seen at a university hospital or its neighborhood clinics.

Appropriate interpretation of urine culture results depends on clinical presentation of the patient and quantitation and identification of cultured organisms. However, reporting of urine culture results varies among commercial and hospital-based clinical microbiological laboratories. Most commercial microbiology laboratories use quantities of 10⁴ CFU/ml as the cutoff for identification of a potential uropathogen and consider any organism isolated at a lower colony count as nonsignificant (5). The UWMC Clinical Microbiology Laboratory’s methods detect, quantify, and report bacteria, if present, as low as 10² CFU/ml (using a 10-µl loop) and identify species samples with ≥10⁴ CFU/ml of a potential uropathogen. If a single organism is present at <10⁵ CFU/ml, the UWMC Clinical Microbiology Laboratory provides a presumptive identification, for example, as a lactose-fermenting Gram-negative rod, and will identify the species if requested by the clinician. Thus, if a uropathogen is present at ≥10⁵ CFU/ml, the UWMC Clinical Microbiology Laboratory provides at least a presumptive identification that is helpful in determining if an organism consistent with a probable uropathogen is present at these low colony counts. This criterion is supported by studies comparing voided midstream urine specimens with bladder catheter-based sampling among healthy, premenopausal women with symptoms of acute, uncomplicated cystitis, demonstrating that colony counts as low as 10⁵ CFU/ml of *E. coli* in voided midstream urine predict bladder bacteriuria (12, 15, 25, 26). Conversely, with the exception of *S. saprophyticus*, the finding of Gram-positive organisms such as group B streptococci or enterococci is rarely associated with bladder bacteriuria in young women (15). Previous studies comparing nonculture diagnostic methods to urine culture results have taken a similar approach with regard to assignment of some results to an indeterminate group (3, 4, 7, 8).

In this study, patient presentation and symptoms were not known for the majority of samples. However, given that the technical basis of the test kit is detection of bacteria, independent of subject symptomatology, it would perform comparably in a clinical study where symptoms are known. RapidBac had a sensitivity of 86% for all significant bacteriuria detected by urine culture (at a level of ≥10⁵ CFU/ml) and a sensitivity of 90% for significant bacteriuria caused by Gram-negative uropathogens. For Gram-negative bacteriuria at ≥10⁵ CFU/ml, RapidBac was highly sensitive at 99%. It is worth noting that most evaluations of other rapid test kits and diagnostic methods have used 10⁴ or 10⁵ CFU/ml as the cutoff for significant bacteriuria by culture, while we have chosen a more stringent but more clinically relevant cutoff of 10⁵ CFU/ml (12, 15, 27, 28). In most commercial laboratories, in order to detect 10⁵ CFU/ml of bacteria, a 1:1,000 dilution of the urine sample is plated, and a positive result constitutes a single colony on the culture plate(s). At this extreme limit of sensitivity, statistical distribution dictates that reproducibility of the culture itself is a limitation to the observed sensitivity and specificity of RapidBac.

The observed specificity of the SLRC test was 94%. However, in samples that were RapidBac positive but culture negative, in approximately 26%, testing in the research laboratory showed evidence of Gram-negative bacteria or fastidious Gram-positive bacteria previously reported as suspected uropathogens in case reports. It has long been suspected that urine culture, while the gold standard for determination of bacteriuria, is not capable of detecting all clinically defined UTIs. Studies have reported culture-negative urine samples in which uropathogens can be detected by PCR (29), and women with symptoms of cystitis whose urine is culture negative usually respond to empirical antibiotic treatment (30).

Urine cultures are frequently ordered for a variety of reasons, including susceptibility testing. However, we found in the present study that most urine cultures produced negative results; to wit, >55% of all cultured samples produced no growth or clinically insignificant mixed Gram-positive growth, and similar results have been reported by others (31). A rapid bacteriuria test kit with high sensitivity and high specificity, and with results available within the time frame of a patient visit, may serve as a useful tool in decreasing the need for expensive and laborious urine cultures by identifying samples likely to produce negative culture results.

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Ann E. Stapleton reports a consultation to Melinta Therapeutics. Mark Geisberg and Robert K. DiNello are employees of the Silver Lake Research Corporation. Thomas M. Hooton reports consultation to Cubist Pharmaceuticals and Melinta Therapeutics and is cofounder of Fimbrion Inc.

REFERENCES


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