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Prospective Evaluation of Light Scatter Technology Paired with Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Diagnosis of Urinary Tract Infections

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ABSTRACT Urinary tract infections are one of the most common reasons for health care visits. Diagnosis and optimal treatment often require a urine culture, which takes an average of 1.5 to 2 days from urine collection to results, delaying optimal therapy. Faster, but accurate, alternatives are needed. Light scatter technology has been proposed for several years as a rapid screening tool, whereby negative specimens are excluded from culture. A commercially available light scatter device, BacterioScan 216Dx (BacterioScan, Inc.), has recently been advertised for this application. Paired use of mass spectrometry (MS) for bacterial identification and automated-system-based susceptibility testing straight from the light scatter suspension might provide dramatic improvement in times to a result. The present study prospectively evaluated the BacterioScan device, with culture as the reference standard. Positive light scatter specimens were used for downstream rapid matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS organism identification and automated-system-based antimicrobial susceptibility testing. Prospective evaluation of 439 urine samples showed a sensitivity of 96.5%, a specificity of 71.4%, and positive and negative predictive values of 45.1% and 98.8%, respectively. MALDI-TOF MS analysis of the suspension after density-based selection yielded a sensitivity of 72.1% and a specificity of 96.9%. Antimicrobial susceptibility testing of the samples identified by MALDI-TOF MS produced an overall categorical agreement of 99.2%. Given the high sensitivity and negative predictive value of results obtained, BacterioScan 216Dx is a reasonable approach for urine screening and might produce negative results in as few as 3 h, with no downstream workup. Paired rapid identification and susceptibility testing might be useful when MALDI-TOF MS results in an organism identification, and it might decrease the time to a result by more than 24 h.

KEYWORDS mass spectrometry, susceptibility testing, urinary tract infection

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Urinary tract infections (UTI) are a leading cause of health care visits in the United States (1). Consequently, urine cultures are one of the most frequently ordered tests in clinical microbiology laboratories (2). Given that culture requires 18 to 24 h for pathogen growth and an additional 18 to 48 h for identification (ID) and antimicrobial susceptibility testing (AST) results, rapid alternatives are needed to streamline therapy. Screening tests, including point-of-care leukocyte esterase and nitrite detection, exist; however, these tests often produce false negatives, particularly in the setting of

low-colony-count bacteriuria (3, 4). More accurate laboratory-based rapid alternatives, including flow cytometry (5, 6) and automated image analysis (7, 8), have been proposed but have not been widely adopted. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been applied as a direct urine-screening tool. Several studies have evaluated this approach using a variety of centrifugation and filtration techniques to separate bacteria from substances that might interfere with MALDI-TOF MS (i.e., white blood cells) (9–17). Unfortunately, these protocols have shown limited promise. The procedures are laborious, and more importantly, they lack sensitivity, with a maximum of 88% sensitivity reported (16), limiting application as a screening tool.

Laser scattering technology has been employed in research, environmental, and food microbiology laboratories for many years and, over 3 decades ago, was initially investigated for urine screening (18). This technology is based on the differential refraction of light by bacterial cells, which is algorithmically interpreted into a growth curve. A commercially available device with a modification of this technology termed narrow-angle forward laser light scattering has recently been reported for the rapid detection of antimicrobial resistance (19). This same device, BacterioScan 216Dx (BacterioScan, Inc., St. Louis, MO), is now advertised for urine screening; positive results suggest bacteriuria, and thus samples should be plated, while negative results can be considered true negatives, without the need for culture. In order for the BacterioScan 216Dx to be adopted clinically, it would need to show close to 100% sensitivity, with a cost-effective specificity. BacterioScan 216Dx has an advertised limit of detection of 10,000 CFU per ml, which is below the threshold for significant bacteriuria by most standards for urine culture, thus making it an attractive screening option. Furthermore, as this device operates via a 3-h incubation of a urine sample diluted in broth medium, investigation into reflex of the resulting suspension directly to MALDI-TOF MS and antimicrobial susceptibility testing is warranted.

The present study prospectively evaluates the performance of the BacterioScan 216Dx device as a urine-screening tool. A subset of screen-positive samples were paired with rapid identification via MALDI-TOF MS and antimicrobial susceptibility testing, potentially reducing standard urine culture turnaround times by greater than 24 h.

RESULTS

A total of 457 urine specimens were prospectively tested on the BacterioScan 216Dx light scatter-based detection instrument. The median age of patients from which specimens were collected was 7 years (interquartile range, 3 to 15 years). Specimens from children less than 90 days old were excluded from the performance analysis ($n = 18$; 3.9%) due to the difference in quantitative criteria for reporting (refer to Materials and Methods). Of the 439 specimens included in the overall performance analysis, 307 (70%) were clean-catch specimens and 132 (30%) were collected by straight catheterization. The vast majority of specimens were chemically preserved ($n = 431$; 98.2%), while a small subset ($n = 8$; 1.8%) were submitted in sterile containers on ice and processed within 2 h.

Conventional urine culture results of these 439 specimens is broken down in Table 1. Of the 439 specimens included in the data analysis, 86 (19.6%) were reported as having significant growth of bacteria, with identification and susceptibility testing performed as appropriate. Of these, 73 (84.9% of culture-positive specimens) had growth of greater than 100,000 CFU/ml, with the majority growing pure *Escherichia coli* ($n = 51$; 59.3% of culture-positive specimens). The overall positivity rate for the forward laser scatter analysis on the same 439 specimens produced 184 positive calls (42%) and 255 negative calls (58%).

Figure 1 shows the comparator culture results for the positive and negative light scatter categories. In total, there were 3 culture-positive specimens that tested as negative by the light scatter device, for a sensitivity of 96.5% (95% confidence interval [CI], 90.1 to 99.3). Two were specimens with greater than 100,000 CFU/ml of *E. coli*. The

TABLE 1 Urine culture and BacterioScan results for specimens included in the light scatter analysis

Organism ID	No. of specimens with indicated result			No. of BacterioScan-negative specimens (no. of CFU/ml)
	10–50K CFU/ml	50–100K CFU/ml	>100K CFU/ml	
Escherichia coli	2	4	51	2 (both >100K)
Klebsiella pneumoniae			3	
Proteus mirabilis		1	2	
Pseudomonas aeruginosa			1	
Streptococcus agalactiae	2	2	2	1 (50–100K)
Staphylococcus saprophyticus			2	
Streptococcus pyogenes			1	
Corynebacterium striatum			1	
Enterococcus faecalis		2	2	
Staphylococcus epidermidis			2	
Gram-positive rod[a]			1	
Mixed			5[b]	
Total (% positive [n = 439])	4 (0.9)	9 (2)	73 (16.6)	3

[a]Unable to identify by routine methods (MALDI-TOF MS, Vitek).

[b]Mixed-culture results were as follows: >100K CFU/ml of *E. coli* and >100K CFU/ml of *E. faecalis* (n = 1), >100K CFU/ml of *E. coli* and 50 to 100K CFU/ml of *E. faecalis* (n = 1), >100K CFU/ml of *E. coli* and 50 to 100K CFU/ml of *Enterococcus avium* (n = 1), >100K CFU/ml of *E. coli* and >100K CFU/ml of *K. pneumoniae* (n = 1), and >100K CFU/ml of *P. aeruginosa* and >100K CFU/ml of *Enterobacter cloacae* (n = 1).

third was a specimen with 50,000 to 100,000 CFU/ml of *Streptococcus agalactiae*. All three were chemically preserved, clean-catch specimens from females aged 15 to 17 years. Growth curves from these samples were reviewed by the manufacturer, with no important differences noted from other negative samples. In total, there were 56 specimens reported as positive by the light scatter device, with no growth reported on culture. There were an additional 45 specimens reported as positive by the light scatter device, with culture results of normal urogenital flora or "mixed" (≥3) organisms. If one considers all of these categories to be false positives, the overall specificity was 71.4% (95% CI, 66.3 to 76.1). Positive and negative predictive values were 45.1% (95% CI, 37.8 to 52.6) and 98.8% (95% CI, 96.6 to 99.8), respectively.

To further evaluate the performance of the instrument, particularly for Gram-positive uropathogens that were underrepresented in the clinical-specimen study, spike-in experiments were performed. Quadruplicate suspensions of *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus aureus* were made in a background of uninfected urine, in four dilutions, spanning the instrument's limit of

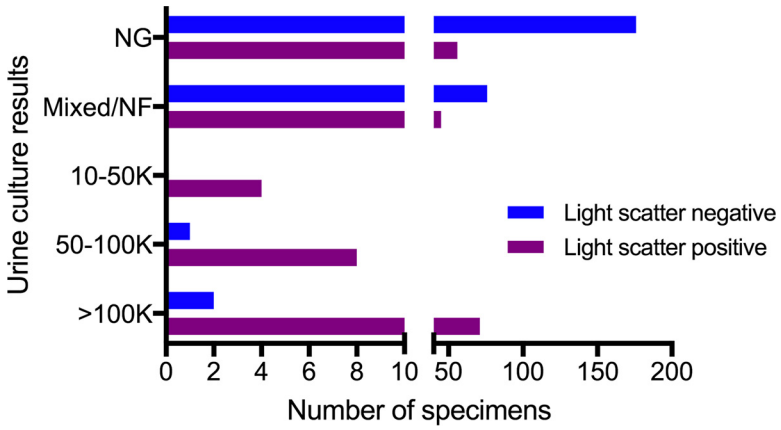

FIG 1 Light scatter results compared with those for the reference standard. Specimens included in the analysis were categorized as either positive or negative by the light scatter device. Corresponding culture results are displayed for each category on the *y* axis, with the number of specimens in each category on the *x* axis. ">100K" refers to >100,000 CFU per ml (and so forth). "Mixed/NF" (where "NF" stands for normal flora) represents cultures with growth that was either ≥3 organisms and considered contaminated or mixed with normal urogenital flora. NG, no growth at 24 h of culture.

TABLE 2 Performance of BacterioScan 216Dx in urine samples spiked with Gram-negative and Gram-positive uropathogens at various densities

Organism or specimen type	Density (CFU/ml)	No. of 216Dx-positive specimens/total
Escherichia coli	1.53×10^6	4/4
	8.33×10^4	4/4
	1.27×10^4	4/4
	1.67×10^3	4/4
Klebsiella pneumoniae	1.87×10^6	4/4
	2.13×10^5	4/4
	1.47×10^4	4/4
	2.90×10^3	4/4
Enterococcus faecalis	1.33×10^6	4/4
	1.00×10^5	4/4
	7.33×10^3	4/4
	1.03×10^3	1/4
Staphylococcus aureus	1.67×10^5	4/4
	1.60×10^4	4/4
	2.10×10^3	2/4
	1.33×10^2	0/4
Unspiked urine	NA[a]	0/4

[a]NA, not applicable.

detection. The performance of the instrument with specimens spiked with Gram-positive organisms was similar to that with specimens spiked with Gram-negative organisms, and 100% of the specimens determined to be positive were above the reported limit of detection of 10,000 CFU/ml (Table 2). Below the instrument's reported limit of detection, Gram-negative uropathogens were more reliably detected (Table 2), suggesting that the instrument may be slightly more sensitive for Gram-negative bacteriuria.

Of the 184 specimens called positive by the light scatter device, the first 58 were used to establish an optical density (OD) cutoff in order to minimize the number of false positives carried through the MALDI-TOF MS protocol (see Materials and Methods and Fig. S1 in the supplemental material). A cutoff of 0.3 McFarland unit, as measured by the densitometer, was determined to increase specificity while maintaining the highest sensitivity. The next 126 positive calls were then evaluated for density (Fig. 2), with 55 (43.7%) samples exceeding the cutoff density of ≥0.3 McFarland unit. The MALDI-TOF MS protocol was carried out on these samples by rapid pelleting of 1 ml of the

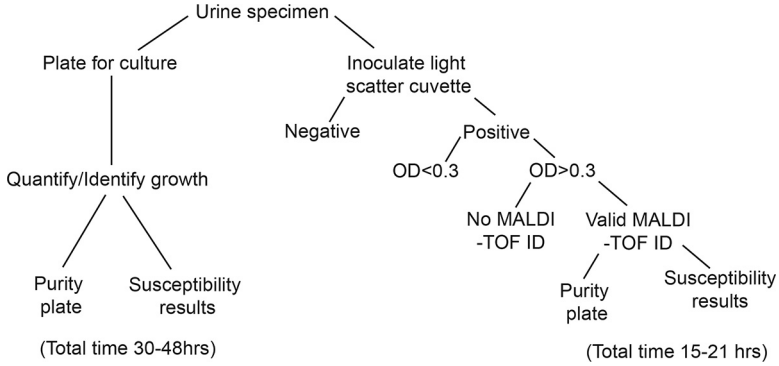

FIG 2 Study design. The reference standard, culture, and workflows are described on the left, with the light scatter, rapid MALDI-TOF MS, and susceptibility testing workflows are described on the right. The total time for the reference standard method is 30 to 42 h, while that for the novel method is 15 to 21 h.

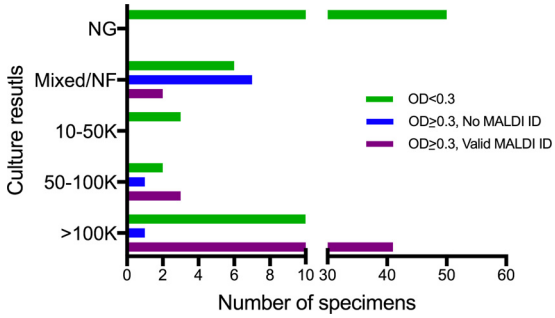

FIG 3 Density-based stratification and MALDI-TOF MS analysis results compared with results for the reference standard. Specimens included in the analysis were categorized based on exceeding the optical density (produced by the densitometer) cutoff of 0.3 McFarland unit and receiving either a valid identification via MALDI-TOF MS (OD ≥ 0.3; valid MALDI ID) or no identification via MALDI-TOF MS (OD ≥ 0.3; no MALDI ID). Specimens that did not exceed the density cutoff of 0.3 McFarland unit were not analyzed by MALDI-TOF MS (OD < 0.3). Corresponding culture results are displayed for each category. ">100K" refers to >100,000 CFU per ml (and so forth). "Mixed/NF" represents cultures with growth that was either ≥3 organisms and considered contaminated or mixed with normal urogenital flora. NG, no growth at 24 h of culture.

suspension incubated in the light scatter device. This pellet was applied to the MALDI-TOF MS target and analyzed by the Bruker clinical-application program.

Figure 3 shows the agreement between the MALDI-TOF MS protocol's identifications and the culture-based identifications. Of the 55 specimens evaluated by the density and MALDI-TOF MS protocol, 46 produced valid species-level identifications, while the remaining 9 had no peaks identified by MALDI-TOF MS. Forty of the 46 valid identifications (87%) corresponded with the correct bacterial species in the setting of a significant monomicrobial culture (Table S1), and 4 IDs (8.7%) distinguished one of the correct bacterial species in a setting of dual uropathogens (Table S1). Two specimens (4.3%) had valid IDs by MALDI-TOF MS but were considered insignificantly mixed by culture analysis. A total of 17 specimens had significant growth by culture but either had no peaks by MALDI-TOF MS ($n = 2$) or were below the density cutoff for MALDI-TOF MS analysis ($n = 15$). The majority of these cultures grew >100,000 CFU/ml of Gram-positive uropathogens ($n = 9$; 53%) (Table S1), consistent with reports showing that identification of Gram-positive bacteriuria by direct-specimen MALDI-TOF MS is less accurate than identification of Gram-negative bacteriuria (14). Taken together, the sensitivity and specificity of this approach were 72.1% (95% CI, 60 to 81.8) and 96.9% (95% CI, 89.5 to 99.5), respectively, with positive and negative predictive values of 95.7% (95% CI, 85.5 to 99.2) and 78.8% (95% CI, 68.6 to 86.3), respectively. Overall, these data suggest that this approach may be useful when a bacterial species is identified by MALDI-TOF MS but should not be used to rule out infection.

All samples with an identification by MALDI-TOF MS ($n = 46$) were used for AST. Of these 46 samples, 2 were not considered significant by culture and thus had no corresponding culture-based antimicrobial susceptibility testing results for comparison, while 4 exhibited mixed infections with 2 different uropathogens. The mixed infections were realized by purity plate analysis; thus, these AST results were also excluded from analysis. The 40 pure samples with corresponding culture-based AST results showed an overall categorical agreement of 99.2% across a 16-drug panel. There were 4 minor errors where the MIC was possibly within 1 doubling dilution and 1 major error where the MIC was discrepant by at least 5 doubling dilutions (Table 3). No very major errors were observed. Overall, this approach was very accurate in diagnosing monomicrobial bacteriuria and reduced the time to AST results by more than 24 h.

DISCUSSION

In the present study, prospective evaluation of the BacterioScan 216Dx light scatter device showed a high sensitivity and negative predictive value, with culture as the reference standard, suggesting that it is a viable approach for urine screening. Imple-

TABLE 3 Antimicrobial susceptibility testing results and noncategorical agreements

Antibiotic(s) tested	No. positive/total no. of specimens tested (% categorical agreement)[a]	Error classification[b]
Ampicillin	39/40 (97.5)	Minor (*E. coli*, *n* = 1, ref R, tested I)
Ampicillin-sulbactam	38/40 (95)	Minor (*E. coli*, *n* = 2, both ref I, tested R)
Piperacillin-tazobactam	39/40 (97.5)	Major (*E. coli*, *n* = 1, ref R, tested S)
Cefazolin	40/40 (100)	
Ceftazidime	40/40 (100)	
Ceftriaxone	40/40 (100)	
Cefepime	40/40 (100)	
Imipenem	40/40 (100)	
Ertapenem	40/40 (100)	
Ciprofloxacin	40/40 (100)	
Levofloxacin	40/40 (100)	
Gentamicin	40/40 (100)	
Tobramycin	39/40 (97.5)	Minor (*E. coli*, *n* = 1, ref I, tested R)
Amikacin	40/40 (100)	
Trimethoprim-sulfamethoxazole	40/40 (100)	
Nitrofurantoin	40/40 (100)	
Total	605/610 (99.2)	

[a]Organisms tested included *E. coli* (*n* = 37), *Proteus mirabilis* (*n* = 2), and *K. pneumoniae* (*n* = 1).
[b]ref, reference method; I, intermediate; R, resistant; S, susceptible.

mentation of this device in our setting would have resulted in a 58% reduction in cultures plated and in labor related to culture reading. Additionally, 58% of specimens submitted for culture would have received a result in around 3 h, compared to the 18 to 24 h required for negative-culture reporting. Pairing the light scatter approach with MALDI-TOF MS resulted in 70.7% of monomicrobial culture-positive samples receiving a correct species-level identification within 3.5 h. Furthermore, reflex of these identifications to antimicrobial susceptibility testing produced highly accurate results in monomicrobial bacteriuria, with the total time from specimen processing to AST results being 15 to 21 h (Fig. 2), compared with 30 to 48 h by culture. Taken together, this reflex approach is a first step to rapid ID and AST results for urinary tract infections.

There are several important limitations to this study. First, our pediatric population had a median age of 7 years, which might make the data difficult to generalize to adult populations. However, our percent culture positivity and distribution of uropathogens were comparable to the same data reported from adult settings (13). Second, the majority of positive urine cultures in this study were monomicrobial infections with *E. coli* (*n* = 57; 70.4% of monomicrobial infections). Thus, our study could not thoroughly evaluate the performance of this device in the setting of Gram-positive bacteriuria (Table 1) (*n* = 16; 19.8% of monomicrobial infections) or other less common Gram-negative rods (*n* = 7; 8.6% of monomicrobial infections). Data from spike-in experiments showed that the instrument may be sensitive for detection of Gram-negative bacteriuria below the instrument's limit of detection but performed equivalently for Gram-positive and Gram-negative uropathogens at >10,000 CFU/ml (Table 2). Third, while the majority of infections in the MALDI-TOF MS and AST analyses were monomicrobial, a small subset (*n* = 3; 4.9% of culture-positive specimens analyzed by the MALDI-TOF MS protocol) were significant bacteriurias with 2 uropathogens. The rapid protocol might identify only mixed infections after analysis of the purity plates. In these cases, after repeat susceptibility testing from isolated colonies, results would require the same amount of time as conventional culture. Fourth, given the prospective nature of the study and the fact that investigators were blind to the results during the study period, we were unable to investigate the 3 false-negative calls by the BacterioScan instrument in real time. As mentioned above, review of growth curves for these samples was unrevealing. It is possible that the discrepancies were the result of manual error whereby the wrong specimens were plated or incorrectly inoculated into the

wrong position in the cuvette, or the barcode was scanned in the wrong position on the cuvette. Fifth, the cutoff set for inclusion in the MALDI-TOF MS protocol was intended to minimize labor wasted on false-positive samples. As 0.3 McFarland unit corresponds with 9×10^7 CFU/ml, the organism density should be roughly 2 logs above the limit of detection of MALDI-TOF MS when a 1-ml pellet is used (20). However, the performance of the MALDI-TOF MS protocol on specimens with McFarland units of slightly less than 0.3 resulted in no peaks obtained (data not shown). Further, several specimens above this cutoff resulted in no peaks obtained (Fig. 3). Thus, the limit of detection of MALDI-TOF MS may be higher than reported depending on the organism identity and growth environment. Finally, given that the majority of positive cultures contained *E. coli* identified by MALDI-TOF MS, a secondary means of confirmation would be required to rule out *Shigella* species, as MALDI-TOF MS systems cannot differentiate the two organisms. While *Shigella* spp. are very unusual urinary tract pathogens, a wet-mount motility experiment from the pelleted suspension could be performed to differentiate the genera.

In addition to the limitations described above, there are several other considerations that laboratories would have to weigh when evaluating the light scatter approach for urine screening. First, we chose to exclude results from children younger than 90 days, as we would not use this device to rule out bacteriuria in this population. However, positive results in children younger than 90 days might be impactful, particularly when paired with results of the rapid MALDI-TOF MS protocol. For example, of the 18 specimens excluded from analysis, one came from a 2-month-old admitted to our hospital under the febrile-infant pathway. The culture grew >100,000 CFU/ml of *E. coli*, and susceptibilities were reported 36 h after collection. Using the light scatter and rapid MALDI-TOF MS approach, a preliminary identification could have been provided in just over 3 h after collection, with susceptibilities reported another 12 to 18 h later, saving almost a day. The second consideration is that 30.4% of specimens called positive by the instrument resulted in no growth. Understanding the characteristics of these specimens might help avoid the additional 3-h delay and expense for a negative urine culture. Cloudy and/or bloody specimens will almost certainly result in a positive light scatter result (per the manufacturer), so labs may choose to plate specimens based on these appearance features. Contamination might also result in false-positive calls, as the cuvettes house up to 4 specimens, possibly leading to incorrect inoculation of open chambers. Third, to maximize cost-effective use, 4 specimens should be inoculated at a time. For laboratories with lower volumes, this may require batching strategies that balance cost with timing, as batching might result in reduced turnaround times if laboratories wait hours for specimens to arrive. Fourth, the manufacturers state that the device is not designed to capture candiduria; therefore, hospitals may have to exclude samples in which the growth of yeasts would be considered significant. Fifth, there is variability from laboratory to laboratory for the criteria used to define significant bacteriuria in straight-catheterization and clean-catch specimens. Laboratories will need to evaluate this technology in the setting of their own significant colony count criteria. Finally, low-level counts of *S. agalactiae* colonies in women of child-bearing age would not be reliably detected by the instrument. While urine culture is not recommended as a screening tool for *S. agalactiae* colonization, laboratories commonly report this information, and it is used to guide prophylaxis (21). Although the BacterioScan 216Dx device is undergoing clinical trials in pursuit of regulatory approval, at the time of this submission, it has not been 510(k) cleared. Thus, it requires a full validation, and data from this study might help guide optimal implementation for all of the discussion points above. Laboratories will need to generate in-house performance characteristics and decide what selective criteria should be used to fit the needs of their population.

As laboratories focus on expanded applications for MALDI-TOF MS, the paired protocol described in this study requires further exploration. The hands-on time was roughly 5 min from cuvette removal to placement of the target in the MALDI-TOF MS instrument, while well-described direct urine-screening protocols require at least 3 spin steps and take roughly 20 to 30 min from sample processing to target loading (10–16).

Unfortunately, rapid identification by MALDI-TOF MS directly from the light scatter suspension was not sensitive enough to detect all culture-positive specimens. The majority of the missed cultures were Gram-positive organisms, and the remaining were Gram-negative rods with 50 to 100,000 CFU/ml reported. This is consistent with reports directly from urine (11, 14) and supported by MALDI-TOF MS's limit of detection of ~100,000 CFU (20). To improve the sensitivity of the rapid MALDI-TOF MS approach, a larger volume of sample could be pelleted or additional hours of incubation could be used to increase optical density; however, the added time of the latter may result in a test that is not more cost-effective than conventional culture. The rapid MALDI-TOF MS protocol identified possible bacteriuria not identified by conventional culture. There were 2 rapid MALDI-TOF MS-positive specimens that were reported in culture as mixed normal flora. One of these was identified via the rapid MALDI-TOF MS protocol as *S. aureus*; however, the culture was reported as mixed normal flora, with no colonies of *S. aureus* identified. The other was an *E. coli* strain identified by rapid MALDI-TOF MS in a child with a positive urinalysis (nitrate-positive) result who was treated with ciprofloxacin for a suspected urinary tract infection despite the culture results. While this case was treated irrespective of microbiologic results, there may be cases in which the light scatter screen paired with rapid MALDI-TOF MS and AST might offer a clinical advantage. Further investigation into the clinical utility of this approach is needed.

MATERIALS AND METHODS

Study specimens. Urine specimens received in our laboratory during weekday dayshifts from August to September 2016 were prospectively tested, with no preference given to urinalysis results or appearance. Samples were excluded from testing if they met any of the following criteria: they were collected via cystoscopy or through suprapubic aspiration, they were preserved in boric acid and received >24 h after collection, they were collected and submitted without preservative at room temperature >30 min after collection, and there was less than 360 μl of sample remaining after routine culture plating. In total, 472 specimens were received during this time period, and 457 were included in the study. Specimens from children less than 90 days old were excluded from analysis, as the significance of growth is interpreted at a lower CFU per ml than the lower limit of detection of the test device. The inclusion of clinical specimens for this study was approved by the institutional review board of the Children's Hospital of Philadelphia.

Urine culture. Routine urine cultures were performed per standard protocols (22). Briefly, a 1-μl loop was used to plate urine samples onto sheep blood and MacConkey agar plates (Remel, Lenexa, KS). Plates were read between 18 and 24 h of incubation at 37°C to assess bacterial growth. Cultures were interpreted according to a standard quantitative analysis. Per our laboratory's protocol, pure growth (or growth of 2 different species) of uropathogens at >10,000 CFU per ml is considered significant in pediatric patients greater than 90 days old from either straight-catheterization or clean-catch specimens. Growth of 3 or more bacterial species was reported as mixed, with no further workup performed. Normal urogenital flora was reported as such. Putative pathogens were identified by MALDI-TOF MS using a Microflex LT (Bruker Daltonics, Billerica, MA), and susceptibility testing was performed via Vitek 2 (BioMérieux, Durham, NC).

Light scatter protocol. The BacterioScan 216Dx instrument is well described in a recent publication by Hayden et al. (19). Urine screening was performed by following the manufacturer's protocol. For this procedure, 360 μl of urine was mixed with 2.5 ml of tryptic soy broth (TSB; Remel) inside the detection cuvette. The cuvettes were loaded directly onto the BacterioScan 216Dx device and continuously read for approximately 3 h. Results were provided as either positive or negative by the device, with no modifications.

Spiked specimens. To determine the performance and dynamic range of the BacterioScan 216Dx's detection of bacteriuria for uropathogens, including Gram-positive organisms that were underrepresented in the clinical study, representative strains of *E. coli*, *K. pneumoniae*, *E. faecalis*, and *S. aureus* were grown overnight in TSB at 37°C with agitation. The resulting cultures were used to prepare suspensions in phosphate-buffered saline (PBS) equivalent to a 0.5 McFarland unit via a DensiCHEK Plus device (bioMérieux, Durham, NC), blanked with PBS. Serial 10-fold dilutions of a culture-negative healthy urine specimen (verified by plate culturing) were then prepared, and each dilution was plated to determine starting bacterial densities. For each pathogen, 360 μl of each dilution was mixed with 2.5 ml TSB in the 216Dx cuvettes, and the instrument was run for 3 h as described above. All bacterial dilutions, along with the corresponding unspiked urine control, were analyzed in quadruplicate.

MALDI-TOF MS protocol. Samples that were resulted as positive by the light scatter instrument were eligible for the MALDI-TOF MS protocol (Fig. 2). After the ~3 h of incubation and analysis, the entire 2.8 ml of the urine and TSB mixture was removed from the detection cuvette via transfer pipette and placed in a 5-ml round-bottom culture tube (VWR, Radnor, PA). This tube was then immediately read by the DensiCHEK Plus device and blanked with TSB, which provides a measurement in McFarland units.

The first 25% of specimens tested on the light scatter device were used to evaluate a density cutoff for MALDI-TOF MS analysis to minimize hands-on time for false-positive specimens. The first 25% of specimens tested resulted in 58 positive calls by the light scatter device. Optical density measurements reported in McFarland units and corresponding culture results from these 58 specimens were used for receiver operating characteristic (ROC) analysis (see Fig. S1 in the supplemental material). The area under the concentration-time curve (AUC) was calculated as 0.829 (95% CI, 0.75 to 0.91). A cutoff of 0.3 McFarland unit was selected for maximum specificity (79%), with no loss in sensitivity (83%).

For samples with a density reading of ≥0.3 McFarland unit, 1 ml was removed and placed in a microcentrifuge tube. This tube was spun for 2 min at a relative centrifugal force (RCF) of 15,700 (13,000 rpm). The supernatant was discarded, and the remaining pellet was immediately touched with a toothpick and spotted directly onto the MALDI-TOF MS steel target. Formic acid overlay and/or extraction was not used during this study, as all pellets tested resulted in either a high-confidence identification or no peaks obtained. The standard Bruker MALDI-TOF MS protocol, including application of matrix and bacterial test standard controls, was followed. The target was then run on the clinical-application program of the MALDI-TOF MS instrument. Results were interpreted according to the manufacturer's specifications.

Antimicrobial susceptibility testing protocol. After MALDI-TOF MS results were obtained, the remaining suspension (1.8 ml) was used to make a 0.5-McFarland-unit suspension via dilution in 0.45% sterile saline (Remel) by following the manufacturer's specifications (Vitek 2; bioMérieux, Durham, NC). The new suspension was then loaded onto the Vitek 2 smart carrier system with the appropriate AST card based on the organism's identity. For this study, only Gram-negative-67 cards were used. A sheep blood agar purity check plate was also streaked for isolation from the 0.5 McFarland unit and read at 12 to 18 h. Only specimens with pure growth on the purity plate were used for downstream agreement analyses. Results were compared with culture AST results, and errors were categorized as minor, major, and very major, as described in reference 23. Briefly, minor errors represent intermediate calls by either the reference standard or the test method, while the opposite method represents susceptible or resistant calls. Major errors represent resistant results by the test method and susceptible results by the reference standard. Very major errors represent susceptible results by the test method and resistant results by the reference standard.

Statistical analysis. ROC and AUC analyses, as well as sensitivity, specificity, and positive- and negative-predictive-value calculations were performed using GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00027-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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