CME/SAM

Diafiltration MALDI-TOF Mass Spectrometry Method for Culture-Independent Detection and Identification of Pathogens Directly From Urine Specimens

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ABSTRACT

Objectives: *With the aim of rapid, culture-independent identification of microorganisms directly from urine specimens, we developed a diafiltration matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method.*

Methods: *In this procedure, urine specimens are desalted, fractionated, and concentrated prior to MS analysis. The analytic performance characteristics of the diafiltration method were assessed in a prospective trial whereby 100 fresh urine specimens were processed using diafiltration MALDI-TOF MS. Concomitant with this, conventional culture was performed with results blinded to the MS operator.*

Results: *The diafiltration method correctly identified urine specimens positive for uropathogens (*Escherichia coli*,* Klebsiella pneumoniae, *and* Proteus mirabilis*) and correctly classified all specimens negative for clinically relevant bacteriuria, including a subset of contaminated urine specimens and a subset with growth of clinically insignificant flora. The sensitivity and specificity of the assay were 67% and 100%, respectively. The detection limit of this method was* 10^5 *to* 10^6 *colony-forming units/mL.*

Conclusions: *Using the diafiltration method, we were able to improve the turnaround time for microorganism identification from 24 to 48 hours (for conventional culture) to 2 to 3 hours. Although methodological refinements are under way to further improve the clinical sensitivity and turnaround time, the 100% positive predictive value of this method suggests that it could be used to guide the selection of antimicrobial agents.*

Upon completion of this activity you will be able to:

- list the screening tests available for diagnosis of urinary tract infections (UTIs).
- discuss the diagnostic performance of screening tests for diagnosis of UTIs.
- describe the workflow for performing a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry test for microorganism identification.

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Questions appear on p 292. Exam is located at www.ascp.org/ajcpcme.

Urinary tract infections (UTIs) constitute a significant health care burden. Uncomplicated UTIs account for six to eight million outpatient visits annually in the United States and 130 to 175 million cases globally.[1,2] In addition, hospital-acquired UTIs comprise approximately 40% of nosocomial infections.[3,4] Although the incidence of UTI over the last decade has remained unchanged, there is an alarming upward trend in antimicrobial resistance in the pathogens isolated from these specimens.[5,6]

In the setting of suspected UTI, physicians rely on a few imperfect screening tests (such as point-of-care dipstick tests for leukocyte esterase, nitrites, and pyuria; microscopy; and/or flow cytometry with automated microscopy) in combination with clinical findings to make a preliminary diagnosis and assess the need to prescribe antimicrobial therapy. Overall, the analytic performance characteristics of these methods are

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poor, and empiric treatment is often recommended in lieu of these screening tests.[7,8] Urine culture (quantitative culture of urine specimens onto solid medium followed by biochemical characterization of isolates) remains the gold standard for diagnosis. The clinical applicability of urine culture–based methods is limited by the fact that they can take 24 to 72 hours before results are available.[9] Considering that a common treatment for an acute uncomplicated UTI is a 72-hour course of antibiotics,[5] culture results may have a limited impact on patient care. Ideally, confirmation of UTI and microorganism identification before the initiation of therapy could help reduce unnecessary use of antibiotics and guide the most appropriate empiric therapy.

The adoption of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in the clinical microbiology laboratory has simplified and expedited microorganism identification. However, MALDI-TOF MS identification methods generally require visible growth on solid media. In the case of UTI, use of MALDI-TOF MS in combination with conventional urine culture can provide improvements in turnaround time (TAT); however, the rate-limiting step is still culture yielding organisms on solid media. To date, there are few examples of MALDI-TOF MS being applied directly to clinical specimens.[10,11] Rapid detection of bloodstream infection is enticing; however, the analytic sensitivity of MALDI-TOF MS precludes application directly to blood specimens because the organism load in bacteremia can be as low as 1 colony-forming unit (CFU)/mL. Therefore, application of MALDI-TOF MS in bloodstream infections requires an amplification step, and MALDI-TOF MS organism identification is limited to positive blood culture broth, where the microorganism(s) concentration is usually at least 10^7 CFU/mL.[12,13]

Urine specimens are more ideal for direct detection because the organism burden for UTI is typically much higher than that for bloodstream infection. The specific organism concentration that defines UTI is the subject of ongoing debate, but most would agree that clinically relevant bacteriuria from a midstream urine specimen occurs at or above 10^5 CFU/mL.[14,15] Most cases of UTI are monomicrobial, obviating the inability of MALDI-TOF MS to resolve polymicrobial specimens.[13] The most common pathogens associated with UTI include *Escherichia coli* (70%-95%), *Staphylococcus saprophyticus* (5%-10%), and other species of *Enterobacteriaceae*, such as *Proteus mirabilis* and *Klebsiella pneumoniae*.[5,9] These pathogens are represented in commercially available MALDI-TOF MS databases. MALDI-TOF MS analysis produces a qualitative result, but we sought to create a method that would identify microorganisms present in urine specimens exclusively at clinically relevant concentrations. Thus, we developed the diafiltration MALDI-TOF MS method to detect and identify uropathogens directly from urine specimens.

Materials and Methods

Materials

High-performance liquid chromatography–grade acetonitrile and formic acid were purchased from Sigma (St Louis, MO) and trifluoroacetic acid was purchased from Fisher Scientific (Pittsburg, PA). Water used in all of the described procedures was obtained from a Milli-Q water purification system (Millipore, Billerica, MA). α-Cyano-4-hydroxy-cinnamic acid matrix (HCCA), bacterial test standard (BTS), and reusable 96-spot stainless steel target plates (MSP 96 target polished steel) were purchased from Bruker (Billerica, MA) and prepared and used according to the manufacturer's instructions. For diafiltration, IVD-labeled centrifugal filters with a 10-kDa molecular weight cutoff were used (Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-10 membrane, Millipore).

Direct Analysis of Unprocessed Samples

An initial analysis was performed whereby liquid samples were evaluated using MALDI-TOF MS without any processing/concentrating steps. Dilutions of *E coli* ATCC 25922 were prepared in sterile water at the following concentrations: 10^8, 10^7, and 10^6 CFU/mL and then applied directly to the MALDI target for analysis. A 2-μL aliquot of each dilution was pipetted onto a MALDI target plate in triplicate, air-dried, and overlaid with 1 μL of matrix before analysis. Each dilution was inoculated onto a blood agar plate in duplicate as a quality control step to confirm colony counts.

Diafiltration Method

Fifteen mL of the urine specimen was transferred into a 15-mL capacity Amicon centrifugal filter **Figure 1**. For particulate-laden specimens exclusively, urine was first centrifuged briefly (30 seconds at 1,000*g*) and the supernatant transferred to the diafiltration device. To concentrate and fractionate the sample, the diafiltration device was centrifuged at 4,000*g* for 25 minutes (or until <1 mL remained in the reservoir) and the flow-through was discarded. To dialyze the sample and lyse RBCs, 12 mL of Milli-Q water was added to the reservoir using a P200 pipette to mix the water with the concentrate in the reservoir. To concentrate and desalt the sample, the filtration device was centrifuged at 4,000*g* for 25 minutes or until less than 1 mL remained in the reservoir. The desalted concentrate was then transferred to a 1.5-mL microcentrifuge tube with a P200 pipette, using the tip to dislodge any sediment from the bottom of the well. The mixture was centrifuged at 14,000*g* for 3 minutes, and the supernatant was carefully pipetted off and discarded. To further desalt the sample, the pellet was resuspended in 1 mL of water and centrifuged again for 3 minutes at 14,000*g*. The supernatant was carefully removed (to not disturb the

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Figure 1 Overview of the diafiltration matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry method, as described in detail in the "Materials and Methods" section.

pellet) with a disposable transfer pipette and discarded. For samples in which the pellet was absent, small, or soft, all but approximately 5 μL of the supernatant was pipetted off, saving the 5 μL of solution for analysis.

Samples were spotted onto a MALDI target plate in duplicate directly from the pellet and/or in a series of dilutions. When the material in the pellet was sufficient, a portion of the pellet was smeared onto the target plate using a wooden toothpick. Pellets were resuspended in an organic solvent solution (50% acetonitrile, 5% trifluoroacetic acid, in water) at set dilutions, and then 1 μL of the suspension was spotted onto the MALDI target plate. Volume for dilution was selected based on the size of the pellet. Small pellets (<10 μL in volume) were diluted in 4 μL of solution, 1 μL was spotted on the target plate, and then 20 μL of solution was added to the sample mixture and again spotted onto the target plate. Larger pellets (>10 μL in volume) were diluted in 20 μL of solution, 1 μL was spotted onto the target plate, and then 80 μL of solution was added to the sample mixture and again spotted onto the target plate. If the mixture was still opaque after the second dilution, the mixture was diluted with 200 μL of solution (and spotted at each dilution step) until the mixture was relatively clear in appearance. In keeping with the standard procedure for MALDI-TOF MS identification of bacterial isolates in our laboratory, 1 μL of 100% formic acid was overlaid over one of the duplicate spots. Once dry, every spot was overlaid with 1 μL of HCCA matrix and left to dry at room temperature before analysis.

MALDI-TOF MS

Spectra were generated and analyzed on the MALDI-TOF Bruker Biotyper microflex LT mass spectrometer using the flexControl operating system and the Bruker Biotyper (version 3.0) software and taxonomy library. MALDI-TOF MS analysis was performed in automatic mode, and a minimum of 240 laser shots were collected for each sample spot. None of the spots on the target plate were reanalyzed or analyzed in manual mode. For each run, BTS was used as a calibrator and as a positive control, and a matrix-only spot was used as a negative control. For identification, the Biotyper software compares the sample spectrum to its database of spectra generated using characterized isolates. All uropathogens discussed herein are represented in this database. Identifications are reported with a confidence score (0-3.0)—a measure of the quality of the match—in which a theoretical perfect match is equivalent to a confidence score of 3.0. Acceptable confidence scores for species-level organism identification had been previously validated at the St Louis Children's Hospital (St Louis, MO) clinical microbiology laboratory, where the MS analysis in this study was performed. Confidence score thresholds compatible with species-level identification were as follows: 1.9 or more for enteric gram-negative bacteria,[16] 1.7 or more for gram-positive bacteria,[17] 2.0 or more for nonfermenting/fastidious gram-negative bacteria, and 2.0 or more for yeasts. In general, according to the manufacturer, a score of 1.7 or more is suitable for genus-level identification. Any organism identified with a confidence score above the aforementioned species-level thresholds were considered high-confidence identifications and were compatible with clinical reporting standards.

Method Development

For method development, the representative uropathogens *E coli* ATCC 25922, *S saprophyticus* (clinical isolate), and *Candida albicans* (clinical isolate) were serially diluted into sterile urine specimens to achieve the following concentrations: 10^7, 10^6, 10^5, and 10^4 CFU/mL. These samples were prepared in preservative-free containers and processed within 15 minutes of preparation (no refrigeration or storage). Three independent replicates of each dilution were tested. Every dilution was inoculated onto the appropriate solid media in duplicate as a quality control step to confirm colony counts.

Prospective Assay Evaluation

One hundred urine specimens were concomitantly analyzed with traditional urine culture (based on the standard operating procedures of the Barnes-Jewish Hospital [St Louis] clinical microbiology laboratory) and the novel diafiltration MALDI-TOF MS method (described herein). All samples included in the study were accompanied by a physician request for a urine culture. Additional inclusion criteria included a urine sample collected in a preservative-free, sterile container and a sample of sufficient volume for both traditional urine culture and diafiltration MALDI-TOF MS analysis. For diafiltration MALDI-TOF MS analysis, an aliquot of the specimen (containing at least 15 mL of urine)

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was made and deidentified for the study team. Urine samples were collected and processed within 2 hours of receipt in either the clinical laboratory or within 2 hours of collection from outpatient clinics (including outpatient testing sites and the obstetrics and gynecology clinic) at Barnes-Jewish Hospital. After performing and analyzing the results of the diafiltration MALDI-TOF MS method, results were compared with those from traditional urine culture, which is considered the gold standard in this study.

After aliquots of patient specimens were taken for initial analyses (diafiltration and conventional culture), if remaining specimen was available, 15-mL aliquots were placed in a preservative-free container and immediately stored at 4°C for potential follow-up analysis. Using known quantities of uropathogens added to sterile urine samples, we verified that storage at 4°C for up to 48 hours in a preservative-free container resulted in no appreciable change in organism concentration in the specimen (by inoculation of samples in duplicate onto solid media).

This study was approved by the Institutional Review Board/Human Research Protection Office of the Washington University School of Medicine (St Louis).

Urine Culture

Conventional urine culture was performed following standard operating procedure for clinical specimens. Briefly, urine was inoculated onto sheep blood agar and MacConkey agar (Remel, Lenexa, KS) using a 1-μL calibrated loop. Subsequent microbial growth was identified using phenotypic methods including Gram stain, Vitek 2, latex agglutination reagents, and other biochemical and spot tests, depending on the organism isolated. All analysis in this study was performed to the same level of resolution used for clinical reporting of these isolates.

Method Comparisons

Dilutions of *E coli* were prepared in sterile urine samples following our protocol used for method development. We evaluated the centrifugation method previously described by Ferreira et al[10] and the filter paper method described by Köhling et al.[11] The centrifugation protocol was performed as previously described using 4 mL of urine[10]; however, with urine samples containing less than 10^7 CFU/mL of microorganisms, we were unable to recover any material (the resultant pellet was miniscule or nonvisible) for transfer onto the MALDI target plate. As such, we modified the final steps in the procedure to mimic those we developed for the diafiltration method by adding a small amount of a matrix-compatible solution (see the “Diafiltration Method” section) to transfer small pellets to the target plate. The filter paper method was performed as previously described.[11] Briefly, 15 mL of urine was filtered through a 0.22-μm Microfil S vacuum filtration device (Millipore). Microorganisms were retrieved from the filter paper by creating a vortex of the filter paper in a microcentrifuge tube with water. The filter paper was then discarded, and organism pellets were formed via high-speed centrifugation and then transferred to the target plate.

Statistics

A one-sample *t* test with a 95% confidence interval (CI) was used to compare the Biotyper scores obtained for a set of dilutions to the target value required for species-level identification. Analyses were performed with IBM SPSS, version 21.0 (Armonk, NY).

Results

Method Development

Direct Analysis of Unprocessed Samples

When dilutions of *E coli* in water were applied directly to the MALDI target plate for analysis (no processing/concentrating steps), an organism concentration of 10^8 CFU/mL or greater was required for identification. No organisms were identified in samples at 10^7 and 10^6 CFU/mL. In this arm of the study, sterile water was used as a diluent to create samples with minimal interfering substances to estimate the lowest concentration at which identification was possible on unprocessed specimens. In light of the very high organism burden required to achieve organism identification, this method was not pursued further.

Detection Limit of the Diafiltration MALDI-TOF MS Method

The detection limit of the diafiltration MALDI-TOF MS method was determined by adding representative uropathogens to sterile urine specimens at known concentrations **Table 1**. For *E coli* and *C albicans,* the detection limit was 10^5 CFU/mL, and for the gram-positive *S saprophyticus* organism, the limit was 10^6 CFU/mL. Using a one-sample *t* test with a 95% CI, we compared the scores obtained at the aforementioned dilutions to the required score for reporting species-level identifications. We found no significant difference between the required score and the sample scores at these dilutions: *E coli* at 10^5 CFU/mL: $t(2) = 0.66$, $P = .58$; *C albicans* at 10^5 CFU/mL: $t(2) = 1.0$, $P = .42$; and *S saprophyticus* at 10^6 CFU/mL: $t(2) = 0.66$, $P = .58$. For gram-positive organisms, the pellet formed during the final step of high-speed centrifugation tended to be less well formed/rigid than that of other organism types and thus more challenging to isolate during the final wash step.

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Table 1
Detection Limit of the Diafiltration Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Method

	Confidence Score[a]			
Pathogen[b]	**10^7 CFU/mL**	**10^6 CFU/mL**	**10^5 CFU/mL**	**10^4 CFU/mL**
Escherichia coli (1.9)	2.3 ± 0.1	2.2 ± 0.2	1.8 ± 0.3	0.9 ± 0.8
Staphylococcus saprophyticus (1.7)	1.8 ± 0.0	1.6 ± 0.3	1.4 ± 0.1	1.3 ± 0.1
Candida albicans (2.0)	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	0.9 ± 0.8

CFU, colony-forming unit.
[a] The mean Biotyper confidence score and standard deviation from three independent experiments.
[b] Known quantities of pathogens were added to sterile urine for analysis. The parenthetical number indicates the required confidence score for acceptable species-level identification for the organism type.

Method Comparison

To compare the diafiltration MALDI-TOF MS with other direct identification methods, we evaluated the detection limit of the centrifugation[10] and the paper-filter[11] methods using *E coli* added to sterile urine specimens **Table 2**. In agreement with another independent evaluation of the centrifugation method,[12] our analysis yielded a detection limit of 10^7 CFU/mL. By modifying the procedure reported by Ferreira et al[10] as described earlier, we were able to improve the detection limit to 10^6 CFU/mL for *E coli* (Table 2). The filter paper method successfully detected uropathogens at 10^7 CFU/mL but not at lower colony counts. In addition to the suboptimal detection limit, the technical challenge of fitting the 4.7-cm diameter filter paper into a 1.5-mL tube was cumbersome. Detection limits for both the centrifugation and filter paper methods paralleled the CFU/mL of the culture-positive urine specimens they were able to reliably identify with MALDI-TOF MS in their respective retrospective trials.[10,11] In a comparison of all three methods, the diafiltration method had superior sensitivity and was the only method capable of identifying organisms at 10^5 CFU/mL (Table 2).

Table 2
Detection Limits for the Diafiltration, Centrifugation, and Filter Paper Methods for *Escherichia coli*

	Confidence Score[a]		
Method	**10^7 CFU/mL**	**10^6 CFU/mL**	**10^5 CFU/mL**
Diafiltration	2.3 ± 0.1	2.2 ± 0.2	1.8 ± 0.3
Centrifugation[10]	1.5 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
Centrifugation-modified[b]	2.4 ± 0.0	1.9 ± 0.3	1.4 ± 0.1
Filter paper[11]	2.5 ± 0.1	1.5 ± 1.3	0.0 ± 0.0

CFU, colony-forming unit.
[a] The mean Biotyper confidence score and standard deviation from three independent experiments. A score ≥1.9 constitutes an acceptable species-level identification.
[b] The centrifugation method was modified by adding solvent to help transfer the pellet to the target plate.

Prospective Trial

Clinical Specimens

A prospective trial of 100 urine specimens submitted to the laboratory for culture was conducted to assess the analytic performance of the diafiltration method. Of 100 specimens processed, 15 had clinically relevant bacteriuria (defined as ≥10^5 CFU/mL with conventional methods). The remaining 85 "negative" specimens included 38 specimens with no bacterial growth, 13 contaminated specimens (that is, specimens containing ≥3 bacterial morphotypes >50,000 CFU/mL), and 34 specimens with clinically insignificant flora (specimens with growth <50,000 CFU/mL). This sampling is reflective of the overall trends seen in urine specimens in the clinical laboratory.

The diafiltration method correctly classified all 85 specimens as negative for clinically relevant bacteriuria, and identified 10 of the 15 UTI-positive specimens **Table 3**. The organisms identified with the diafiltration method included six specimens that yielded *E coli*, three *K pneumoniae*, and one *P mirabilis*. Of these, one specimen (*E coli*) had a colony count exceeding 10^7 CFU/mL. No incorrect or false-positive results were seen. This method resulted in a sensitivity of 67%, specificity of 100%, negative predictive value (NPV) of 94%, and positive predictive value (PPV) of 100%.

Table 3
Prospective Trial of 100 Urine Specimens Submitted to the Clinical Laboratory for Culture[a]

	Conventional Analysis	
	+	–
Diafiltration method		
+	10	0
–	5	85[b]

+, clinically relevant bacteriuria; – no growth, insignificant growth, or contaminated with mixed flora.
[a] Specimens were evaluated using the diafiltration matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method compared with conventional analysis.
[b] Negative specimens included 38 specimens with no growth of organisms, 13 "contaminated" urine specimens, and 34 specimens with growth of clinically insignificant flora.

When the results were deemed to be falsely negative relative to conventional analysis, the diafiltration method was repeated on refrigerated-specimen aliquots **Table 4**. Sufficient quantities were available to repeat analyses for four

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Table 4
Results of Repeat Analysis of False-Negative Specimens With the Diafiltration Method

Organism Identification	Conventional Analysis (CFU/mL)	Repeat Diafiltration Analysis (Confidence Score)
Escherichia coli	10^6	QNS
E coli	$>10^7$	*E coli* (2.0)
Serratia marcescens	10^6	*S marcescens* (1.8)
Coagulase-negative *Staphylococcus*	10^5	No identification
Viridans group *Streptococcus*	10^5	No identification

CFU, colony-forming units; QNS, quantity not sufficient.

of the five specimens that had false-negative findings with the diafiltration method. In two specimens, the microorganisms were not identified on repeat analysis. The colony counts for these specimens (10^5 CFU/mL) fall into the low-probability zone of identification via the diafiltration method (Table 1). The two specimens that were correctly identified on repeat analysis provided useful information regarding methodological improvements. *Serratia marcescens* was readily identified at the genus level. The *E coli* specimen ($>10^7$ CFU/mL) formed a very large pellet during processing. In the repeat analysis, species-level identification was made on both a pellet spot and the second dilution spot. The high particulate concentration of this sample likely interfered with ionization and/or database matching. Because of the turbidity of the sample, several additional dilutions were made, but confidence scores declined, suggesting that in addition to diluting the interfering substance(s), we also diluted the concentration of the microorganism beyond the detection capabilities of the method. In general, for large pellets (greater than the volume of approximately 10 μL of solvent) and particulate-laden specimens, we recommend continuing dilutions of the pellet until the 1-μL aliquot spotted on the MALDI target plate is almost clear in appearance. This procedure should ensure that samples are spotted in a concentration range amenable to MALDI-TOF MS analysis while also attempting to dilute any interfering substances.

Turnaround Time

The TAT of the diafiltration method—time of receipt in the clinical laboratory to pathogen identification—was approximately 2 to 3 hours, depending on the number of clinical specimens being processed simultaneously (Figure 1). No technician time is required for most of this processing time: two 25-minute centrifugation steps and 30- to 60-minute automated MS analysis.

Discussion

This report describes the development and evaluation of a novel diafiltration method for rapid, culture-independent microorganism identification via MALDI-TOF MS. The assay was developed with a targeted detection limit of 10^5 CFU/mL, and its analytic characteristics were evaluated in a prospective fashion using an unbiased set of urine specimens from patients with suspected UTIs.

During evaluation of the detection limit of the diafiltration MALDI-TOF MS method, known concentrations of *E coli, S saprophyticus*, and *C albicans* were added to sterile urine specimens. The diafiltration method was able to reliably detect all of the aforementioned organisms at 10^6 CFU/mL, as well as *E coli* and *C albicans* at 10^5 CFU/mL. Consistent with analyses of direct detection methods for positive blood cultures,[18,19] we achieved higher identification rates for specimens containing gram-negative organisms relative to gram-positive organisms. This may be partly due to cell wall differences between the two organism types, with gram-positive bacteria being more resistant to cell lysis, and, in some circumstances, to the higher bacterial titers associated with gram-negative vs gram-positive infections.

In lieu of urine, other published methods have used either sterile saline or water for their detection limit studies[10,20]; one method did not report a formal evaluation of the detection limit of their method.[11] Because urine is a complex biological matrix, sterile saline or water cannot adequately mimic the potential interferences in urine specimens and may overestimate the performance characteristics of the method. We therefore systematically compared the diafiltration method with other published methods using sterile urine as the diluent. The diafiltration method described herein had superior sensitivity to the previously published methods.

The diafiltration method also had robust specificity (100%). This is particularly important in the context of UTIs in which a relatively large proportion of the specimens received in the clinical laboratory will not represent clinically relevant bacteriuria. The diafiltration method achieved the desired specificity during the preliminary analyses; no species- or genus-level identifications were reported for samples with concentrations below 10^5 CFU/mL or for contaminated urine specimens. Each of the samples below the cutoff for clinically relevant bacteriuria either did not generate a spectrum or the spectrum generated did not result in organism identification. Thus, we were able to show that a low abundance of contaminating flora does not interfere with the diafiltration MALDI-TOF MS analysis.

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Candida was included in the preliminary analysis despite being clinically controversial because it is recovered from urine with conventional culture, especially in hospitalized patients. To reliably detect *Candida* using the diafiltration method, the formic acid overlay step after application of the sample to the MALDI target plate was essential. Fortunately, the formic acid overlay does not have a negative effect on the confidence score or rate of identification of other microorganisms and is used routinely in our clinical laboratory.[16,17]

After the detection limit was evaluated and methods were compared, the diafiltration method was subsequently evaluated using specimens submitted to the clinical microbiology laboratory for culture. Adding uropathogens in known quantities to sterile urine is an idealized means to initially evaluate urine-processing strategies for downstream MALDI-TOF MS analysis, but it does not adequately represent the variety of urine specimens received in the clinical laboratory. Urine specimens from patients with suspected UTI vary considerably in composition. Significant variability is seen in the concentration of proteins, small molecules, lipids, particulates, and cells (WBC, RBC, epithelial, etc) and in the resultant pH, specific gravity, osmolality, and turbidity. Thorough assessment of direct identification methods for UTI requires the analysis of an unbiased set of clinical specimens. Previously reported evaluations of other methods have been retrospective and have preselected for primarily monomicrobial culture-positive specimens.[10,11] Such analyses do not address method specificity because specimens that could result in false-positive results (specimens with organisms in clinically insignificant amounts or contaminated with mixed flora) are excluded. In contrast, our study was conducted on an unbiased sampling of clinical specimens to determine analytic performance relative to the gold standard, urine culture.

Based on the results of the prospective trial (PPV = 100%), the diafiltration MALDI-TOF MS method can be used to rule-in UTI in a clinical setting. Although the sensitivity of the method (67%) suggests that improvements in the detection limit are warranted, particularly for gram-positive organisms, the method had excellent specificity (100%). In addition to direct comparison with urine culture, the results of diafiltration can also be compared with the current rapid testing method, urine dipsticks, which have modest sensitivity (61%-93%) and specificity (63%-78%).[21] The diafiltration MALDI-TOF MS method had superior specificity with at least comparable sensitivity, but this is difficult to gauge because of the wide range of sensitivities reported for dipstick testing. A key consideration when comparing these rapid testing methods is that the diafiltration method specifically identifies the causative pathogen.

Reanalysis of four false-negative samples was helpful in identifying areas of improvement for future method refinement. Two of the falsely negative specimens, a viridans group *Streptococcus* and a coagulase-negative *Staphylococcus*, contained organisms at concentrations below the detection limit determined in the preliminary analysis (Table 1). These two taxa are unlikely to be clinically significant because they are rarely uropathogenic in individuals with normal urinary anatomy, with the exception of individuals with indwelling medical devices. Nevertheless, these organisms were identified in the urine culture, and our aim was to use the diafiltration method to detect and identify organisms present. These false-negative specimens suggest that modest improvements in sensitivity would further increase the correlation with urine culture. The other two false-negative specimens (containing *E coli* and *S marcescens*) yielded correct identifications on reanalysis. Because the uropathogens in these specimens were at concentrations amenable to detection, it is likely that ion suppression (because of the unusually high particulate content of the *E coli* specimen) and technical error (in the case of the *S marcescens* specimen) were responsible for the initial missed identifications.

Like conventional identification methods used for organisms, the diafiltration method does not provide antimicrobial susceptibilities; therefore, antimicrobial susceptibility testing (AST) may still be needed. Although existing guidelines recommend that AST be performed when organisms are detected in urine (above various thresholds), the correlation between in vitro susceptibility breakpoints and in vivo conditions in urine has not been established.[9] Susceptibility breakpoints are based on bloodstream concentrations for antimicrobials and have not been correlated to urine (where many agents are significantly more concentrated), limiting the need for AST in the setting of UTI.[9] For cases of UTI in which the responsible microorganism is rapidly identified, physicians can turn to institutional antibiograms to quickly refine antimicrobial therapy selections. Akin to direct detection MALDI-TOF MS methods for bloodstream infections, the goal of the diafiltration method is to rapidly detect UTI and identify the organism as close as possible to the point of care.

Our study has some limitations. During the prospective analysis of urine samples, we had few positive findings (15 of 100 specimens) and there was limited diversity in the organisms represented. Due to the unbiased specimen inclusion criteria, the number of true-positive findings accurately reflects the prevalence of positive cultures in our population (~15%). Similarly, the organisms identified are reflective of the typical organism distribution observed for UTIs.[5,9] The method required 15 mL of urine, and we only evaluated specimens received in preservative-free urine cups; as such, the performance characteristics of other specimen types are unknown. Our study also has some unique strengths. We systematically compared the detection limit of previously

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reported methods and our novel method in the representative sample matrix, and our study did not have specimen selection bias toward known positive specimens in the prospective trial. Previous studies preselected samples with known culture or biochemical characteristics, such as enriching for positive samples or using only specimens with high bacterial counts as determined on culture.[10,11,22] Such an approach is limiting, as it precludes the determination of basic assay characteristics such as PPV or NPV and results in unknown performance on the most common specimen types encountered in the clinical laboratory (no growth, "contaminated," and insignificant growth specimens). In assessing a diagnostic method for UTI, both sensitivity and specificity are clinically important to ensure appropriate use of antimicrobial therapy.

Using the diafiltration method we were able to improve the TAT for microorganism identification from 24 to 48 hours (for conventional culture) to 2 to 3 hours. With respect to implementation in the clinical laboratory, it should be noted that the most time-consuming steps of the diafiltration method require no hands-on time (two 25-minute centrifugations and a 30- to 60-minute MALDI-TOF MS automated identification). The remaining steps are amenable to automation, which could further improve TAT and improve workflow. The cost per test, including reagents and filtration device, was approximately $10. Ultimately, the implementation of direct identification protocols should be weighed against the clinical benefit. For instance, the implementation of direct detection methods from positive blood culture bottles has demonstrated that the clinical usefulness and impact of these methods outweighs potential drawbacks from hands-on processing time and increased costs.[23,24] In the context of UTIs, the considerable improvement in TAT achieved with the diafiltration method moves UTI detection and identification into a clinically desirable timeframe.

The ability to identify a pathogen in a urine specimen within 1 hour of receipt in the clinical laboratory has the potential to expedite targeted antimicrobial therapy and reduce unnecessary antibiotic use. With this long-term goal in mind, methodological refinements are currently under way to further improve the clinical sensitivity and TAT of the diafiltration MALDI-TOF MS method.

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References

1. Vincent C, Boerlin P, Daignault D, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg Infect Dis*. 2010;16:88-95.
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med*. 2002;113(suppl 1):5-13.
3. Klevens RM, Edwards JR, Richards CL, et al. Estimating health care-associated infections and deaths in US hospitals, 2002. *Public Health Rep*. 2007;122:160-166.
4. Haley RW, Culver DH, White JW, et al. The nationalwide nosocomial infection rate: a new need for vital statistics. *Am J Epidemiol*. 1985;121:159-167.
5. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis*. 2011;52:e103-e120.
6. Mehnert-Kay SA. Diagnosis and management of uncomplicated urinary tract infections. *Am Fam Physician*. 2005;72:451-456.
7. Schwartz DS, Barone JE. Correlation of urinalysis and dipstick results with catheter-associated urinary tract infections in surgical ICU patients. *Intensive Care Med*. 2006;32:1797-1801.
8. Perkins J, Perkins K, Vilke GM, et al. Is culture-positive urinary tract infection in febrile children accurately identified by urine dipstick or microanalysis? *J Emerg Med*. 2012;43:1155-1159.
9. Burd EM, Kehl KS. A critical appraisal of the role of the clinical microbiology laboratory in the diagnosis of urinary tract infections. *J Clin Microbiol*. 2011;49(suppl):S34-S38.
10. Ferreira L, Sánchez-Juanes F, González-Ávila M, et al. Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*. 2010;48:2110-2115.
11. Köhling HL, Bittner A, Müller K-D, et al. Direct identification of bacteria in urine samples by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and relevance of defensins as interfering factors. *J Med Microbiol*. 2012;61:339-344.
12. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev*. 2012;36:380-407.
13. DeMarco ML, Ford BA. Beyond identification: emerging and future uses for MALDI-TOF in the clinical microbiology laboratory. *Clin Lab Med*. 2013;33:611-628.
14. Kwon JH, Fausone MK, Du H, et al. Impact of laboratory-reported urine culture colony counts on the diagnosis and treatment of urinary tract infection for hospitalized patients. *Am J Clin Pathol*. 2012;137:778-784.
15. Coulthard MG, Kalra M, Lambert HJ, et al. Redefining urinary tract infections by bacterial colony counts. *Pediatrics*. 2010;125:335-341.

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16. Ford BA, Burnham C-AD. Optimization of routine identification of clinically relevant gram-negative bacteria using MALDI-TOF MS and the Bruker Biotyper. *J Clin Microbiol.* 2013;51:1412-1420.
17. TeKippe EM, Burnham C-AD. Optimizing identification of clinically relevant Gram-positive organisms using the Bruker Biotyper MALDI-TOF MS system. *J Clin Microbiol.* In press.
18. Buchan BW, Riebe KM, Ledeboer NA. Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. *J Clin Microbiol.* 2012;50:346-352.
19. Meex C, Neuville F, Descy J, et al. Direct identification of bacteria from positive anaerobic BacT/Alert blood cultures by MALDI-TOF MS: MALDI Sepsityper kit (Bruker) versus in-house saponin method for bacterial extraction. *J Med Microbiol.* 2012;61:1511-1516.
20. Wang XH, Zhang G, Fan YY, et al. Direct identification of bacteria causing urinary tract infections by combining matrix-assisted laser desorption ionization-time of flight mass spectrometry with UF-1000i urine flow cytometry. *J Microbiol Meth.* 2013;92:231-235.
21. Deville WL, Yzermans JC, van Duijn NP, et al. The urine dipstick test useful to rule out infections: a meta-analysis of the accuracy. *BMC Urol.* 2004;4:4.
22. Ferreira L, Sánchez-Juanes F, Muñoz-Bellido JL, et al. Rapid method for direct identification of bacteria in urine and blood culture samples by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: intact cell vs. extraction method. *Clin Microbiol Infect.* 2011;17:1007-1012.
23. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Arch Pathol Lab Med.* 2013;137:1247-1254.
24. Clerc O, Prod'hom G, Vogne C, et al. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. *Clin Infect Dis.* 2013;56:1101-1107.

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