SUNDAY- 254

Utilization of Laser Light Scattering for Rapid Detection and Subsequent Antimicrobial Susceptibility Testing Directly from Urine Specimens

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Abstract

Background

The high volumes and lengthy turnaround times required to process urine specimens present a workflow challenge and prompt the use of empiric therapy, including to patients whose specimens may be uninfected. Methods that return diagnostic information more rapidly would improve patient care and facilitate antibiotic stewardship efforts. The BacterioScan 216Dx is a laser light scattering device that detects urinary tract infections (UTIs) more rapidly than current standard of care (SOC) methods. Our lab evaluated the 216Dx's ability to provide rapid antimicrobial susceptibility testing (AST) data using samples deemed positive during the UTI screening protocol.

Methods

Ninety-five urine specimens were analyzed using the 216Dx UTI detection protocol according to the manufacturer's instructions. Cuvette contents from positively flagged specimens were spread onto pre-warmed blood and MacConkey agar plates and incubated at 37°C for 4 hours. MALDI-TOF MS (Bruker) identification was performed on the resulting agar surface film. Direct-from-positive specimen AST was also evaluated on 22 samples containing Gram negative pathogens using a panel of 4 antibiotics tested at clinical breakpoint concentrations. Cuvette contents were diluted to ~5x10^5 CFU/ml into cation-adjusted Mueller Hinton Broth prior to dispensing into antibiotic-containing cuvettes. AST runs in the 216Dx were conducted at 35°C for 16 hours, after which optical profiles were compared to a corresponding no drug control.

Results

Fifty-four specimens possessed ≥10,000 CFU/ml of a UTI pathogen, 51 of which were accurately detected by the 216Dx after 190 minutes. Despite contaminant presence in many of the specimens, MALDI-TOF MS analysis of positively-flagged cuvette material showed ~75% agreement with SOC data, with the majority of failed samples harboring lower density infections, Gram positive pathogens, or possessing >1 UTI pathogen. The SOC AST method (MicroScan) indicated non-susceptibility for 36.3, 36.3, 4.5, and 0% of isolates to Ciprofloxacin (CIP), Levofloxacin (LVX), Cefepime (FEP), and Meropenem (MER), respectively. After 3-4 hours of analysis, the 216Dx demonstrated categorical agreement at 100% for CIP, FEP, and MER, and 95.4% for LVX.

Conclusions

Based on these data, the BacterioScan 216Dx has the potential to offer accurate UTI detection and, when coupled with MALDI-TOF MS, robust pathogen ID/AST for at least 75% of Gram negative infected urine specimens, within as little as 8 hours post-collection. Further validation is needed and ongoing.

Background

One of the primary strategies being implemented in hospitals to combat the spread of antimicrobial resistance is antimicrobial stewardship, and guidelines and practices promoting the appropriate use of antibiotics have been developed by institutional antibiotic stewardship committees. The effective implementation of these practices is inhibited by the lengthy time-to-result requirements of conventional diagnostic platforms. Antimicrobial susceptibility testing can take 24-48 hours, and results are often available as long as 3-5 days after initial specimen collection. While awaiting results, clinicians must use empiric antibiotic strategies, which facilitate the emergence of resistant pathogens and may negatively impact the morbidity and mortality of critically ill patients. New diagnostic platforms that can provide antimicrobial susceptibility testing (AST) results in a more timely manner have potential to improve patient care by optimizing antimicrobial therapy at an earlier time in the patient's treatment course. Ideally, new assays could provide diagnostic information regarding bacterial identification and antimicrobial susceptibility directly from the specimen.

Narrow angle forward laser scattering is a sensitive optical method for measuring particles suspended in liquid (Figure 1), and can be used to monitor changes in microbial densities at levels well below thresholds detectable with conventional methods (i.e. traditional spectrophotometry, unaided eye). The BacterioScan 216Dx™ is a Laser Microbial Growth Monitor that can deliver precise assessments of microbial populations in liquid samples, and has the ability to provide automated measurements during long-term incubation at a desired temperature (i.e. 37°C). The instrument measures the density of bacteria by measuring changes in both light scattering properties and optical density, enabling a limit of detection as low as 1x10^4 CFU/ml, which is about twice the background level for purified water. The 216Dx uses a disposable multicuvette that holds up to four individual samples, each containing up to 5.0 ml each, and can hold four multicuvettes at a time for a total of up to sixteen samples per instrument run. The system is random access, allowing multicuvettes to be added or removed at any time.

The 216Dx was originally designed and engineered to rapidly screen urine specimens to detect urinary tract infections (UTIs) and to eliminate uninfected samples from the culture-based workflow. This qualitative test application recently received 510(k) clearance from the FDA. While earlier stratification of negative urine specimens improves clinical laboratory workflow and antimicrobial avoidance, the additional benefits of more rapidly identifying infected specimens were also considered. Utilizing the 216Dx's incubation capability while urine specimens are being analyzed for positivity/negativity, the growing contents within each multicuvette slot were hypothesized to serve as a reasonable substrate from which further pathogen information could be obtained. Specifically, the potential downstream applications of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for infecting organism identification, as well as rapid, phenotypic AST profiling were speculated to be achievable directly from 216Dx-positive UTI samples. In this study, we evaluated this comprehensive process using clinical urine specimens and compared the performance of laser light-scattering methods to those obtained using conventional approaches.

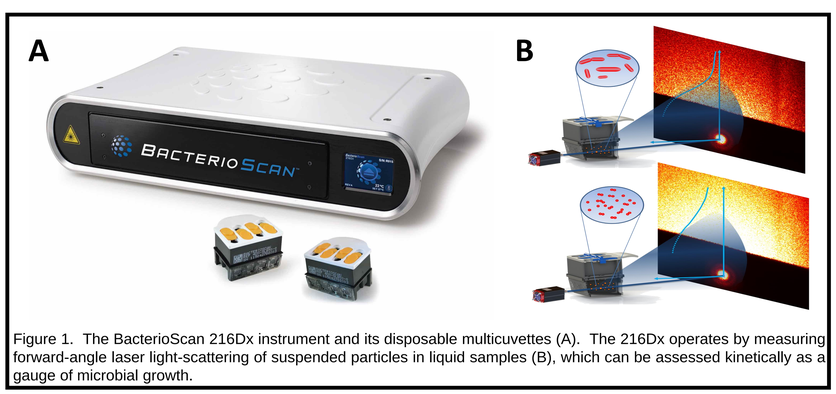
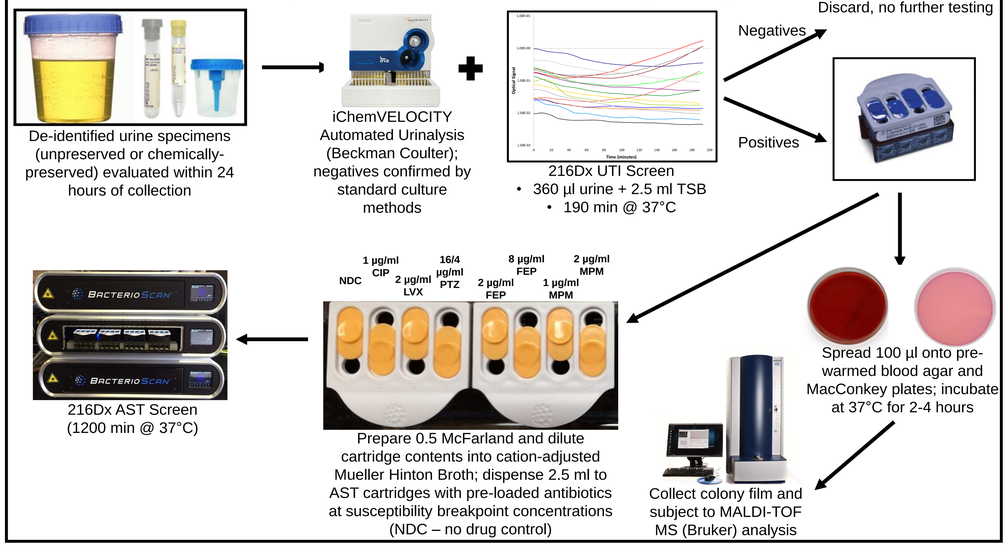


Figure 1. The BacterioScan 216Dx instrument and its disposable multicuvettes (A). The 216Dx operates by measuring forward-angle laser light-scattering of suspended particles in liquid samples (B), which can be assessed kinetically as a gauge of microbial growth.

Experimental Methods and Study Design



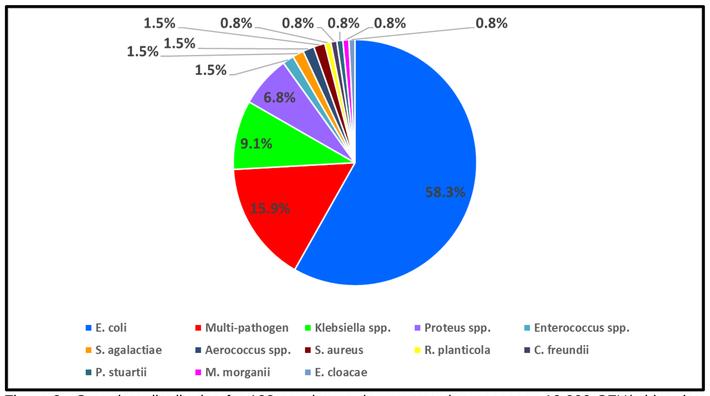


Figure 2. Organism distribution for 132 specimens demonstrated to possess ≥10,000 CFU/ml by plate culturing.

Table 1. Comparative performance of the BacterioScan 216Dx and iChemVELOCITY automated urinalysis instrument (UA) for detection of bacterial presence (stratified by bacterial density) in patient urine specimens.

	≥10,000 CFU/ml		≥50,000 CFU/ml		≥100,000 CFU/ml	
	216Dx	UA	216Dx	UA	216Dx	UA
True Positives	121	126	113	116	107	106
True Negatives	160	119	162	119	167	120
False Positives	56	97	64	107	70	117
False Negatives	11	6	9	6	4	5
Total samples	348	348	348	348	348	348
Sensitivity	91.7%	95.5%	92.6%	95.1%	96.4%	95.5%
Specificity	74.1%	55.1%	71.7%	52.7%	70.5%	50.6%
PPV	68.4%	56.5%	63.8%	52.0%	60.5%	47.5%
NPV	93.6%	95.2%	94.7%	95.2%	97.7%	96.0%
Accuracy	80.7%	70.4%	79.0%	67.5%	78.7%	64.9%

Acknowledgments

All reagents, instruments, and technical guidance were provided to Loyola University Medical Center by BacterioScan at no cost.

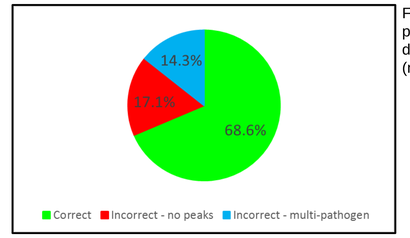


Figure 3. UTI pathogen identification performance from MALDI-TOF MS using direct-from-positive-specimen processing (n=105).

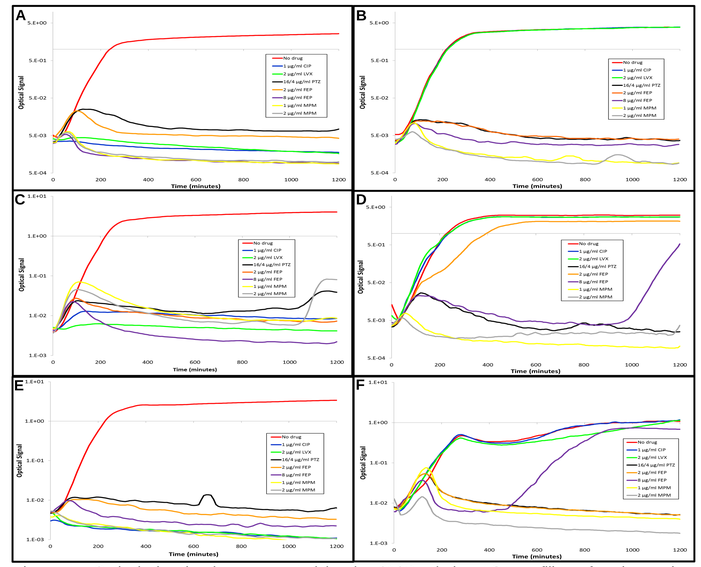


Figure 4. Optical signal values generated by the 216Dx during AST profiling of various urine specimens. After being classified as UTI positive during the 216Dx urine screening run, cuvette contents were withdrawn and diluted into cation-adjusted Mueller Hinton Broth before being dispensed into antibiotic pre-loaded cartridges. Specific specimens evaluated included those that harbored a pan-susceptible *E. coli* (A), a fluoroquinolone-resistant *E. coli* (B), a pan-susceptible *K. pneumoniae* (C), a fluoroquinolone-resistant, ESBL-producing *E. coli* (D), a pan-susceptible *E. cloacae* (E), and a fluoroquinolone-resistant *P. mirabilis* (F). All AST results were in agreement with those obtained using standard reference methods (MicroScan).

Table 2. Incidence of antibiotic resistance and corresponding 216Dx direct-from-positive-specimen AST performance for 38 infected urine specimens.

Antibiotic	% strains resistant/susceptible	Categorical Agreement
Ciprofloxacin	36.8/63.2	100%
Levofloxacin	34.2/65.8	97.4%
Piperacillin-Tazobactam	7.9/92.1	89.5%
Cefepime	5.3/94.7	100%
Meropenem	0/100	100%

Conclusions

- Laser light-scattering can be a useful method for screening clinical urine specimens for the presence or absence of bacteria.
- The 216Dx UTI detection protocol demonstrates comparable sensitivity and substantially greater specificity when compared to automated urinalysis.
- Using an abbreviated incubation and surface film for bacterial growth, MALDI-TOF MS-based identification provided accurate and rapid identification of organisms for ~70% of the samples when compared to standard culture. Samples that did not provide accurate results using the rapid MS method either harbored multiple organisms or started as low density infections in the initial specimen. No instances of inaccurate pathogen identification were observed.
- Phenotypic AST profiling of five standard-of-care UTI antibiotics in the 216Dx resulted in categorical agreement values of ≥90% when tested directly from positively-flagged UTI samples. These results were available after 2-4 hours of optical analysis (depending on the growth rate of the organism).
- These results support the development of a single process that utilizes laser light-scattering to discriminate between infected and uninfected urines and provide pathogen characterization information in a fraction of the time conventional methods require.