SUNDAY- 425

Process Development for the Rapid Detection and Characterization of Infected Urine Specimens Using Laser Light-Scattering

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Abstract

Background
The processing of urine specimens requires overnight incubation to determine positivity, thus more rapid methods would facilitate antibiotic stewardship efforts by reducing the unnecessary use of antibiotics in uninfected patients. The ability to reliably and more rapidly perform antimicrobial susceptibility testing (AST) on infecting pathogens would also improve patient care considerably. The use of laser light-scattering, exemplified by the BacterioScan 216Dx instrument, has recently been explored for these purposes and a comprehensive assessment of clinical urine specimens was performed to evaluate its utility.

Methods
A total of 254 unpreserved or preserved human urine specimens were evaluated using the 216Dx 3-hour UTI application. Samples identified as positives were processed (1) for viable counts, both pre- and post-216Dx analysis; (2) for mass spectrometry evaluation using an aliquot of the post-run cuvette contents; (3) for rapid AST evaluation in the 216Dx comparing both direct-from-specimen (DFS) and direct-from-isolate (DFI) inputs, using susceptibility breakpoint concentrations of 7 antibiotics. CLSI-compliant minimum inhibitory concentration (MIC) values were generated and served as references for comparative purposes.

Results
76 urines were determined to possess ≥10,000 CFU/ml of a uropathogen. The 216Dx had a UTI detection sensitivity and specificity of 95% and 56%, respectively. The median starting density of all positive urines was $5.0x10^7$ CFU/ml, which increased by an average of 265-fold after processing in the 216Dx. Mass spectrometry analyses of post-run cuvette contents demonstrated a robust identification capability for all positive specimens with densities ≥$1x10^6$ CFU/ml. When compared with MIC values generated using standard methods, both DFS and DFI rapid AST results from the 216Dx were highly correlative for ciprofloxacin (98.1% categorical agreement), trimethoprim-sulfamethoxazole (96.2%), nitrofurantoin (90.4%), ceftriaxone (100%), cefazolin (96.2%), ampicillin (96.2%), and fosfomycin (92.3%). The time-to-result, which was determined based on comparisons to individual no-drug controls, ranged from 1.8-4.6 hours for both DFS and DFI inputs.

Conclusions
Laser light-scattering provided a rapid and effective screening method for urine specimens with clinically-relevant densities of uropathogens. Processing in the 216Dx also enabled reliable pathogen identification using mass spectrometry, and subsequent DFS AST strongly predicted susceptibility/resistance.

Background

Bacterial and fungal infections constitute very serious and very constant threats to human health, claiming the lives of thousands of people on an annual basis. Further complicating matters is the worsening imbalance between the development of antimicrobial resistance and of new antimicrobial agents, forcing clinicians to use combinations of broad-spectrum antibiotics or those with known toxicity liabilities. Unfortunately, each of these therapeutic strategies has the potential to worsen patient condition and outcome. As a consequence, guidelines promoting the more prudent use of antibiotics have been mandated by institutional antibiotic stewardship committees. The implementation of these practices is still restricted by the lengthy time-to-result requirements of conventional diagnostic platforms. The process of definitively determining pathogen resistance/susceptibility can take 24-48 hours beyond isolation of the organism (totally 3-5 days from initial specimen collection), which is time critically-ill patients do not have. As a consequence, clinicians resort to empiric antibiotic use, which facilitates the emergence of resistant pathogens. Thus the need for new diagnostic platforms that can provide antimicrobial susceptibility testing (AST) results with a more expedient turn-around-time is greater than ever. In particular, those assays which can provide preliminary predictions of resistance/susceptibility directly from patient specimens should demonstrate the most substantial impact, as the time required to culture isolated colonies of the infecting pathogen would be eliminated from the process.

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 4 individual samples, each containing up to 3.0 ml each, and can hold 4 multicuvettes at a time for a total of up to 16 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 serve the purpose of rapidly screening urine specimens as a means to detect urinary tract infections (UTIs) and to eliminate uninfected samples from the culture-based workflow. Representative performance values for this clinical application, which requires 3 hours to provide a qualitative result, are shown in Table 1. While earlier stratification of negative urine specimens should improve clinical laboratory workflow and enable antibiotic stewardship campaigns, the additional benefits of more rapidly identifying infected specimens were also considered. Leveraging 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.

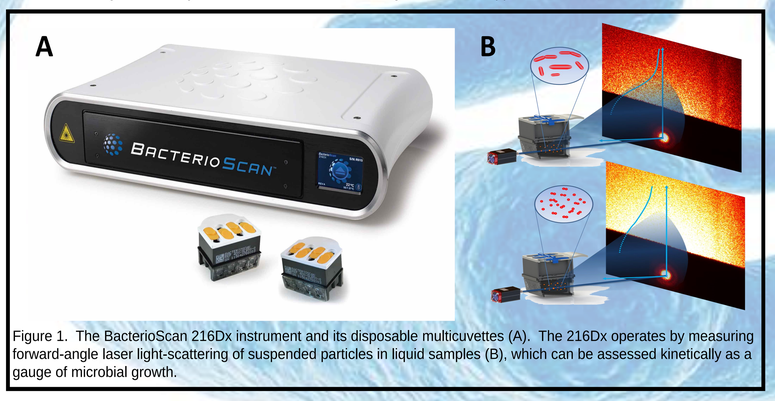


Figure 1. The BacterioScan 216Dx instrument and its disposable multicuvettes (A). The 216Dx operates by measuring forward-angle laser light-scattering of suspended particles in liquid samples (B), which can be assessed kinetically as a gauge of microbial growth.

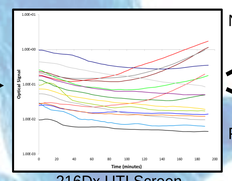
Table 1. Multisite performance characteristics of the BacterioScan 216Dx UTI screening protocol using a pathogen density threshold of 10,000 CFU/ml.

Clinical Study	Number of Specimens	Incidence (% pos.)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Plate Savings (%)
Study 1	296	37.8	91.1	73.4	67.5	93.1	80.1	45.6
Study 2	253	29.3	91.9	76.0	61.3	95.8	80.6	53.8
Study 3	329	25.2	91.6	66.7	48.1	95.9	73.0	49.9
Study 4	197	44.7	93.9	72.2	70.6	94.3	81.2	42.1
Study 5	228	46.1	96.3	65.8	61.2	97.0	76.8	42.1
Study 6	232	13.8	96.9	68.5	33.0	99.3	72.4	59.1

Experimental Methods and Study Design

DAY ONE: Direct-from-specimen (DFS)

De-identified urine specimens (unpreserved or chemically-preserved) evaluated within 24 hours of collection



216Dx UTI Screen
- 360 µl urine + 2.5 ml TSB
- 190 min @ 37°C

Negatives → Discard, no further testing

Positives →

216Dx AST Screen (1200 min @ 37°C)

Dilute cartridge contents into cation-adjusted Mueller Hinton Broth; dispense 2.5 ml to AST cartridges with pre-loaded antibiotics at susceptible breakpoint concentrations (ND – no drug control)

Prepare 1 ml aliquot (centrifuge, aspirate, wash) for MALDI-TOF MS analysis

Quantify bacterial density pre/post 216Dx analysis

DAY TWO: Repeat 216Dx AST using isolated colonies as inocula (Direct-from-isolate; DFI) targeting $5x10^4$ and $5x10^5$ CFU/ml; perform reference AST using broth microdilution and Vitek2 (bioMerieux)

Table 2. Performance characteristics of the 216Dx UTI screening protocol using a pathogen density threshold of 10,000 CFU/ml (left), and the reference information for the six false negatives identified.

# specimens	318*
Incidence	29.9%
Sensitivity	93.7%
Specificity	56.1%
PPV	47.6%
NPV	95.4%
Accuracy	67.3%
Plate savings	39.3%

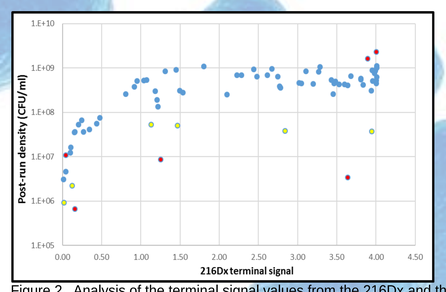
Organism	Storage	Density (CFU/ml)
E. coli	CP	10-50,000
E. faecalis	UNP	10-50,000
S. agalactiae	CP	50-99,000
S. agalactiae	UNP	10-50,000
S. agalactiae	CP	50-99,000
S. agalactiae	CP	50-99,000

* Two *Candida glabrata* specimens excluded from analysis

Table 3. UTI pathogen breakdown, density quantifications, and mass spectrometry-based identification performance for 82 positive urine specimens.

Pathogen	Number of Specimens (% of total)	Average/median initial density (CFU/ml)	Average/median density post-216Dx (CFU/ml)	MALDI attempts	MALDI successes	No peaks detected	MALDI score range[b]
Escherichia coli	62 (62.9)	$1.48x10^8/6.80x10^7$	$5.33x10^8/5.00x10^8$	57	53	4	2.01-2.44
Streptococcus agalactiae	9 (8.3)	$5.60x10^5/5.60x10^5$	$1.03x10^7/1.03x10^7$	2	1	1	2.31-2.36
Klebsiella spp.	6 (6.2)	$3.52x10^8/4.92x10^7$	$3.85x10^8/2.95x10^8$	6	4	2	2.26-2.42
Proteus mirabilis	4 (4.1)	$1.68x10^7/1.68x10^7$	$5.68x10^8/5.68x10^8$	4	3	1	2.40-2.51
Enterobacter spp.	4 (4.1)	$2.60x10^6/3.48x10^8$	$2.04x10^7/4.60x10^8$	4	4	0	1.79-2.45
Pseudomonas aeruginosa	4 (4.1)	$2.31x10^7/2.25x10^7$	$1.26x10^8/5.95x10^7$	3	1	2	2.36-2.41
Enterococcus faecalis	4 (4.1)	$9.06x10^5/2.30x10^5$	$8.37x10^7/4.90x10^7$	3	2	1	2.18-2.49
Citrobacter spp.	2 (2.1)	$3.30x10^8/3.30x10^8$	$3.60x10^8/3.60x10^8$	2	1	0[c]	2.41-2.51
Staphylococcus saprophyticus	1 (1.0)	ND[a]	ND[a]	0	0	0	NA
Staphylococcus aureus	1 (1.0)	$3.20x10^5/3.20x10^5$	$2.20x10^6/2.20x10^6$	1	0	1	NA

[a] – ND – not determined
[b] - all MALDI-TOF MS analyses were performed in duplicate; NA – not applicable
[c] - an incorrect identification was generated



Sample processing guidelines targeting $2.5x10^5$-$7.5x10^5$ CFU/ml

- **If average of final 3 216Dx datapoints are >0.5, dilute 1:1000**
- **If average of final 3 216Dx datapoints are 0.1-0.5, dilute 1:100**
- **If average of final 3 216Dx datapoints are <0.1, dilute 1:10**

Figure 2. Analysis of the terminal signal values from the 216Dx and the resulting bacterial densities after execution of the UTI screening protocol suggests a correlative relationship that can be used to guide sample preparation methods for DFS AST. Applying the rules established (right), the majority of samples (blue points) would yield densities that were within 0.5 $\log_{10}$ units of the target range, whereas a few would be ~1.0 $\log_{10}$ units (yellow) or >1.0 $\log_{10}$ units (red) from the target range.

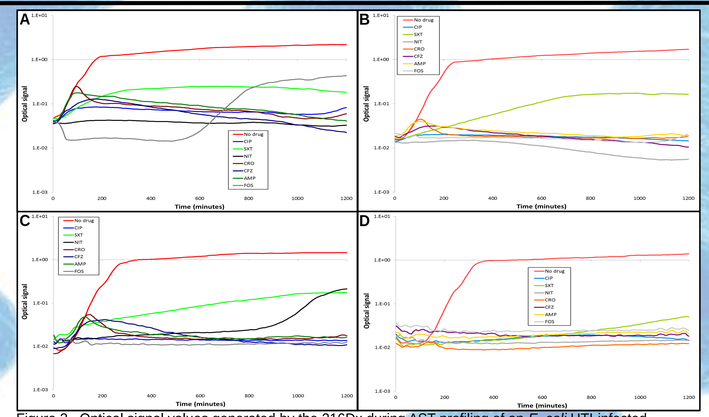


Figure 3. Optical signal values generated by the 216Dx during AST profiling of an *E. coli* UTI-infected specimen. After being classified as UTI positive during the 216Dx urine screening run, cuvette contents were withdrawn and diluted 1:250 (A) or 1:2500 (B), resulting in starting DFS densities of $3.0x10^6$ and $3.0x10^5$ CFU/ml, respectively. Additionally, isolated colonies that were recovered after 24 hours of blood agar plate culturing were used to prepare DFI suspensions of $5.0x10^5$ (C) and $5.0x10^4$ (D) CFU/ml. All AST results were in agreement with those obtained using standard reference methods.

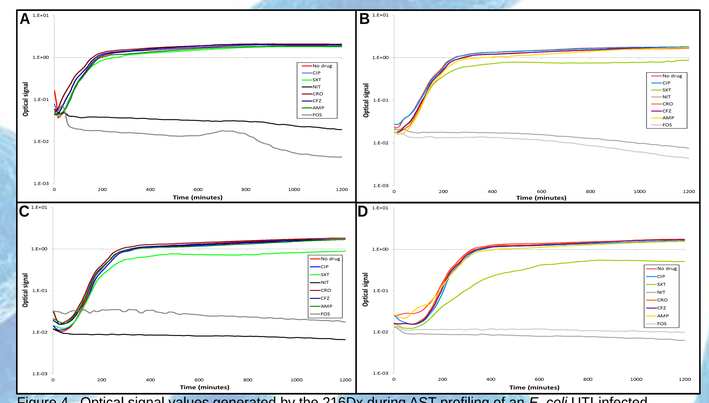


Figure 4. Optical signal values generated by the 216Dx during AST profiling of an *E. coli* UTI-infected specimen. After being classified as UTI positive during the 216Dx urine screening run, cuvette contents were withdrawn and diluted 1:250 (A) or 1:2500 (B), resulting in starting DFS densities of $2.5x10^6$ and $2.5x10^5$ CFU/ml, respectively. Additionally, isolated colonies that were recovered after 24 hours of blood agar plate culturing were used to prepare DFI suspensions of $5.0x10^5$ (C) and $5.0x10^4$ (D) CFU/ml. All AST results were in agreement with those obtained using standard reference methods.

Table 4. Categorical agreement values representing 216Dx AST performance using DFS (n=82) and DFI (n=74) samples.

Antibiotic	% strains resistant/susceptible	DFS 1:250 dilution	DFS 1:2500 dilution	DFI $5x10^5$ CFU/ml	DFI $5x10^4$ CFU/ml
Ciprofloxacin	17.1/82.9	98.8	98.8	97.3	97.3
Trimethoprim-sulfamethoxazole	26.8/73.2	90.2	91.4	91.9	93.2
Nitrofurantoin	19.5/80.5	92.7	93.9	91.9	91.9
Ceftriaxone	15.9/84.1	96.3	97.6	98.7	98.7
Cefazolin	18.3/81.7	96.3	95.1	94.6	94.6
Ampicillin	48.8/51.2	93.9	93.9	97.3	97.3
Fosfomycin	8.5/91.5	91.5	91.5	90.5	90.5

Conclusions

- Laser light-scattering can be a useful method for rapidly profiling clinical urine specimens for UTI positivity/negativity.
- UTI screening performance in this study was fairly consistent with that demonstrated across multiple clinical sites previously.
- Through the incubation functionality of the 216Dx, UTI pathogen populations were observed to increase by an average of 1-2 $\log_{10}$, which proved beneficial for downstream characterization processes.
- MALDI-TOF MS-based identification of UTI pathogens was demonstrated to yield accurate information for 98.6% of the samples in which sufficient proteomic signals were obtained. The majority of specimens in which no peaks were detected had initial densities <$1x10^6$ CFU/ml.
- Phenotypic AST profiling of 7 standard-of-care UTI antibiotics in the 216Dx resulted in categorical agreement values of >90% when tested both DFS and DFI. These results were available after 2-6 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.

Acknowledgements

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