Sunday - 412
June 4, 2017

Utility of Laser Scattering Technology followed by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for Urinary Tract Infection (UTI) Screening and Identification

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Introduction

Urine culture is often performed in high volume with many negative samples that require 18 – 24 hours for results. Screening urine samples for organism prior to culture may reduce this volume and provide more timely information. Current technology utilizing narrow angle forward laser scattering via the 216 Dx device (BacterioScan) is designed to flag urine samples with microbial growth characteristics at >10^4 CFU/ml as "likely positive" with a three hour time to results.

MALDI-TOF MS (Bruker) has dramatically improved the time required to identify common organisms. Direct identification from urine samples is feasible if organisms can be recovered in high enough number. The ability to reliably screen for the presence of organisms at quantities >10^4 CFU/ml and subsequently perform MALDI-TOF MS organism identification (ID) were explored in this pilot study. Methods to distinguish negative urine samples from potential positives and to rapidly identify organisms associated with urinary tract infection (UTI) are presented.

Methods

Urine Samples

Submitted for urine culture from adult patients (>18 yrs)
Collected <24 hours prior to testing

- **2.5 ml Tryptic Soy Broth + 360 µl urine mixed in cuvettes**
  (2 cuvettes + additional tube for phase 1; single cuvette for phase 2)
- **Incubated for 3 hours at 37ºC with growth automatically monitored**
- **Semi-quantitative urine culture inoculated and incubated for 24 hours**
  (<10^4 CFU/ml = negative; 3 or more organisms = contaminated)

BacterioScan (Bscan) Positive Cuvettes

2 ml removed from cuvette, centrifuged and pellet washed in 1 ml water

Phase 1 Processing:

Duplicate cuvette + tube held at 37ºC for 2 additional hrs.

Direct smear (DS) of 3 hr pellet applied to MALDI target
Identification score ≥1.99 – no further processing

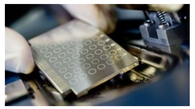
Score <1.99 – repeat DS on 5 hr incubated cuvette
Identification score ≥1.99 – no further processing

Score <1.99 – perform extraction (EX) processing (ethanol followed by formic acid and acetonitrile)

Phase 2 Processing:

If large pellet, MALDI direct smear performed

If small pellet, MALDI extraction performed



Results

- 224 samples were tested; 151 under phase 1 processing and 73 in phase 2.
- Urine cultures were positive (68; 30.4%), negative (91; 40.6%) or contaminated samples (65; 29%).
- BacterioScan screening results (Figure 1) were positive (124; 59.3%) or negative (85; 40.7%) with (15) discordant duplicate samples (10.2%).
- BacterioScan sensitivity, specificity, positive and negative predictive values were 95.5, 57.8, 51.6, 96.5, respectively with culture as gold standard (Table 1).
- 44 Gram-negative pathogens had DS MALDI-TOF ID, score ≥1.99 at 3 hours (Table 2).
- Gram-positives had DS (6) or EX (4) identification score ≥1.99, or no reliable ID (4).
- Yeast were identified by MALDI-TOF EX score ≥1.99 (1) or no reliable ID (4).
- Culture negative-false positives had no MALDI-TOF ID (22/23) and 1 *K. pneumoniae.*
- Contaminant culture-false positives had no reliable ID (21), EX (8) or DS (8) MALDI-TOF ID, score ≥1.99. Four DS ID were after extended incubation.
- BacterioScan false negative screens (3) occurred with *E. faecalis, M. morganii* and *S. mitis/oralis* organisms (Figure 2).

Table 1: BacterioScan Screening Results Compared to Gold Standard Urine Culture

Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
95.5	57.8	51.6	96.5
Urine Samples (224)	**BacterioScan Positive (124)**	**BacterioScan Negative (85)**	**BacterioScan Discordant (15)**
Urine Culture Positive (68)	64	3	1
Urine Culture Negative (91)	23	60	8
Contaminated Urines (65)	37	22	6

Table 2: MALDI-TOF Organism Identification of BacterioScan Positive Cuvettes (Score value ≥ 1.99)

	Direct Smear ID	Extraction ID	Low or No ID
Bscan Positive/Culture Positive (64)			
Gram-Negative Organisms (45)	*E. coli* (30)		*E. coli* (1)
	K. pneumoniae (5)		
	P. mirabilis (2)		
	E. aerogenes (1)		
	Mixed GN (6)		
Gram-Positive Organisms (14)	*E. faecalis* (2)	*E. faecalis* (2)	*Lactobacillus* (1)
	E. faecium (1)	Mixed GP (2)	*S. aureus* (1)
	Lactobacillus (1)*		No ID (2)
	S. simulans (1)		
	Mixed GP (1)		
Yeast (5)		*C. albicans* (1)	*C. albicans* (1)
			C. glabrata (1)
			No ID (2)
Bscan Positive/Culture Negative (23)	*K. pneumoniae* (1)*		No ID (22)
Bscan Positive/Contaminated (37)	*Corynebacterium* (1)	*C. glabrata* (1)	*E. faecalis* (1)
	E. coli (3)**	*E. faecalis* (1)	*Gardnerella* (1)
	K. pneumoniae (1)*	*E. faecium* (1)	*Lactobacillus* (2)
	K. oxytoca (1)	*Lactobacillus* (2)	*P. aeruginosa* (1)
	M. morganii (1)*	*M. morganii* (1)	No ID (16)
	P. stuartii (1)	*S. anginosus* (1)	
		Mixed GP (1)	

* MALDI-TOF ID after 5 hour incubation ** 1 ID at 3 hours; 2 after 5 hour incubation

Figure 1:

Growth curves of eight culture positive urines with duplicate BacterioScan positive screening, shown as solid and dashed lines.

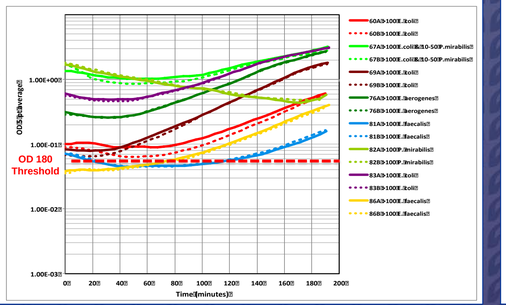
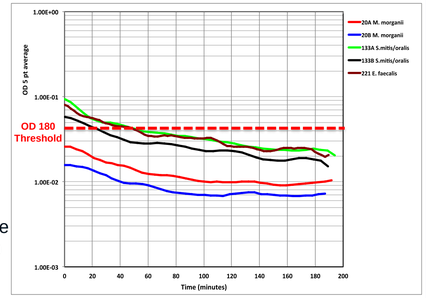


Figure 2:

Three culture positive urines did not meet BacterioScan positive criteria of OD 0.043 at 180 minutes or 15% or more increase in slope from 100 to 180 minutes.



Conclusions

- Negative urines can be reliably screened out using the 216 Dx BacterioScan device to reduce unnecessary cultures.
- Most Gram-negative and Gram-positive organisms producing a BacterioScan positive screen result can be rapidly identified by MALDI-TOF direct smear.
- Many BacterioScan false positive screen results can be discerned by lack of, or low score, MALDI-TOF identification.
- Urine culture remains necessary to determine if samples are polymicrobic or contaminated and to perform antimicrobial susceptibility testing.

Acknowledgements

We gratefully acknowledge BacterioScan Inc. for providing the 216 Dx device, cuvettes, broth, training and technical support for our evaluation. We also acknowledge and thank Amber Garavaglia who served as honest broker providing de-identified urine samples and reports for the study.