

Evaluation of BiesseBioscreen as a new methodology for bacteriuria screening

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SUMMARY

Urinary tract infection is a common disease diagnosed from symptoms and clinical signs, and bacterial count per volume of urine. This study have evaluated the BiesseBioscreen analyzer as a new way to analyze urine samples enabling fast screening of urine, prior to reference standard methods currently utilized in microbiology analysis laboratory. We analyzed 962 urine samples from outpatients and inpatients of the Tor Vergata (TV) University Hospital of the University of Rome "Tor Vergata". All samples were processed both with the BiesseBioscreen and with the standard methodology adopted by the clinical microbiology laboratory of TV Hospital and the results were compared. Of the samples analyzed 54.9% were concordant negative with the reference method and 21.6% concordant positive, 23.3% resulted false positive and 0.2% false negative. The results obtained from BiesseBioscreen showed a sensitivity of 99.0%, indicating it as a system suitable to rule out urinary tract infection. BiesseBioscreen could represent a valid method for screening negative samples to exclude from culture test with a potential reduction in time, workload and costs of the diagnosis.

KEY WORDS: Bacteriuria screening, Fluorescent assays, Urinary tract infection, Nucleic probe, Bacterial culture.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections, in both hospitalized and community patients and its diagnosis requires symptoms, signs and the urinoculture results. These last data are obtained in the clinical microbiology laboratory (Peleg *et al.*, 2010; Stamm *et al.*, 1993).

For most patients the disease of the infection is minimal but for particular subpopulations may develop serious complications. Urine culture is the standard for diagnosing urinary tract in-

fections, even if it is a laborious procedure and produces more than 60% of negative results (Broeren *et al.*, 2011; Brilha *et al.*, 2010; Jolkonen *et al.*, 2010).

A fast screening method able to reduce the urine culture will have a deep impact on laboratory in term of workload and cost for a clinical analysis. A screening method able to identify and to exclude negative urine samples with a high sensitivity and high negative predicted value, to avoid positive urine samples from being classified as negative, would have a large impact on laboratory economics.

Moreover the exclusion of bacteria infection through a fast test may help to reduce unnecessary prescriptions and usage of antibiotics. The increased usage of antibiotics indeed has consequences, such as prolonged infections, that may dramatically increase recovery time, hospital stays and health care costs. Further-

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more it may also determine the growth of resistant micro-organisms which do not respond to conventional treatment, resulting in prolonged illness and greater risk for health.

Our aim is to evaluate the detection of bacteria by BiesseBioscreen, in order to identify negative urine samples, excluding them from urine culture. BiesseBioscreen is an instrument developed by ASI (Milan, Italy) based on a technology patented in collaboration with the University of Urbana-Champaign, IL, USA, (patent number: US7, 973, 294 B2; Jul. 5, 2011). The BiesseBioscreen method described in the patent, allows measuring the concentration of fluorescent particles in a liquid medium (Magde *et al.*, 1974; Chen *et al.*, 1999a; Chen *et al.*, 1999b; Duda *et al.*, 2001; Berland *et al.*, 1995; Digman *et al.*, 2008). The instrument is equipped with a rotating and translating sample holder. BiesseBioscreen uses pattern recognition data analysis techniques for measuring the concentrations and for characterizing fluorescent particles on the basis of size, shape, diffusion constant and or composition.

In this study we compared the detection of bacteria of BiesseBioscreen to the standard methodology adopted by the clinical microbiology laboratory of Polyclinic Tor Vergata, University hospital of the University of Rome Tor Vergata. The laboratory adopts the HB&L-URO4 Alifax: a kit for a rapid automated bacteriuria screening and for residual antimicrobial activity (RAA) testing in urinary samples, with results obtainable in 3 hours. (Ballabio *et al.*, 2010; Fortina *et al.*, 2010; Barocci *et al.*, 2010; Ilki *et al.*, 2010; Tessari *et al.*, 2010; Milagro *et al.*, 1999).

The system uses light scattering technology to detect the growth of bacteria and has been recently utilized for the culture of fluid samples, for the detection of antibiotic-resistant bacteria, and for antibiotic-susceptibility testing directly on urine samples. (Roveta *et al.*, 2004; Roveta *et al.*, 2006; Fontana *et al.*, 2009).

MATERIALS AND METHODS

Patients

A total of 962 urine specimens were collected from both inpatients and outpatients of the

Tor Vergata (TV) University hospital of Rome "Tor Vergata". The collection was done in two different periods: 481 samples in September 2012 and 481 in March 2013. We collected all the routine samples, sampled by the microbiology laboratory in the periods indicated, with no selection. Each sample was identified by a serial code with no patient information such as sex or age. The microbiology screening were performed using the HB&L-Uro4 system by the hospital Clinical Microbiology Laboratory, and using the BiesseBioscreen system by the Department of Experimental Medicine and Surgery of the University of Rome "Tor Vergata".

Microbiological analysis with the HB&L-Uro4 system

The HB&L-Uro4 test gives information of live bacteria count reported in CFU/ml, using a patented technology based on light scattering measurements.

A laser scans sample holder and the detected signals are translated into growth curves. A total of 500 μ l of sample was seeded in a 2 ml broth vial, placed in the machine incubation box at 37°C and monitored for 6 h.

The samples were read every 5 minutes. A magnetic stirrer bar homogenized the vial content before each reading, and growth of microorganisms was monitored in real-time by displaying growth curves on the computer screen. Furthermore, the HB&L-Uro4 system is also programmed, via the residual antimicrobial activity (RAA) test, to evaluate the RAA in the samples at the time of screening, detecting the residual antimicrobial activity of a drug administered to a patient before the collection of specimens.

The system employs two vials, a gold-capped vial used for the culture test and a red-capped vial for RAA test. Both vials contain the enriched medium intended to support the growth of the majority of microorganisms. Upon arrival in the laboratory, 500 μ l aliquots of each fluid sample were dispensed in the culture and RAA vials. The RAA vials were inoculated with the reference strain derived from *Staphylococcus epidermidis* ATCC 12228 according to the supplier's instructions. The samples were cultured in the HB&L-Uro4 for 6 hours to achieve a cut-off lower than 50 cfu/ml.

Instrument characteristics

The present instrument comprises an optical analysis device for determining the concentration of particles in a fluid sample. It has a cylindrical cuvette (about 1 cm in diameter) for holding the fluid sample, with two motors that provide a rotational (about 2 rev/s) and a slower vertical inversion (about 2.5 centimeters/s) motions. The slow vertical scanning is useful to ensure statistical independence of the observation volume explored in subsequent vertical sweeps. The excitation light is generated by a 532 nm neodymium-yttrium laser.

A lens focuses the excitation light onto the sample held in the cuvette thereby causing particles in the sample to generate fluorescence, and also collects the fluorescence from an observation volume in the sample.

The illumination focus is centered about 200 μm from the wall of the cuvette inside the sample. The photodetector is in optical communication with the confocal microscope and receives a portion of the fluorescence from the observation volume measuring its intensity as a function of time, thereby generating a temporal profile of the fluorescence from the observation volume.

The combination of a confocal microscope and a means for moving the container holding the sample provides an effective means for transporting substantial volumes (e.g. milliliters) of the fluid sample through the observation volume without requiring a flowing system.

The processor, having a pattern recognition algorithm is in communication with the photodetector for receiving an output signal corresponding to the temporal profile generated by the photodetector. The pattern recognition algorithm analyzes the temporal profile, determining the concentration of the particles in the samples. The algorithm matches features in the temporal profile to predetermined patterns that correspond to the time-dependent fluorescence intensities of particles passing through the observation volume.

The concentration of particles is determined by calculating the number of predetermined patterns matched to features in the temporal profile for a given sample scanning period. Concentrations are extracted from the analyzed temporal profile by dividing the number of matches by

the volume of sample analyzed during a selected sample scanning period, which can be accurately calculated with knowledge of the size of the observation volume, rate of movement of the container (e.g. rate of vertical and horizontal displacement) and the duration of the sample scanning period.

Microbiological analysis with the BiesseBioscreen system

Analyses with BiesseBioscreen were performed on the same sample processed by the Uro4-HB&L reference method. Samples were stored on ice and examined within 5 hours of receipt in the laboratory. Measurements were performed diluting at first 30 μl of urine samples in 1 ml of isotonic solution and incubating for 7 minutes at 80°C. After dilution, 30 μl 0.05 mM of nucleic fluorescent probe (SYTOX® Orange Nucleic Acid Stain, INVITROGEN c.n.: S11368) were added to the samples. Finally before measuring, the samples were further diluted up to 3 ml. The cuvette containing the 3 ml sample was inserted in the instrument, measured for 60 seconds at the end of which the operator read directly on the instrument screen the value of the CFU/ml. Each sample was measured five times and the average value was used for the statistical analysis.

Whereas the experiment was performed in two distinct periods, after the results obtained in the first run some changes were added in the second run to improve the measurements protocol. In the first testing the 481 samples were diluted in phosphate buffer (PBS), while in subsequent measurements, isotonic solution was used for the other 481 samples. Moreover in the second period of measurements each sample was prepared twice and measured twice to exclude a manual error or a pipette malfunction.

Statistical methods

All analyses were performed in comparison with HB&L-Uro4 that was used as the gold standard. The evaluation was performed considering for both methodologies, a sample as positive if it exceeded the 100,000 CFU/ml. This cut-off value was established according to the guidelines of the Italian Association of Clinical Microbiology (AMCLI). Analogously for the BiesseBioscreen screening, samples

were considered positive if exceeding 100,000 bacteria/ml.

We calculated the Sensitivity (Se) of the BiesseBioscreen as the proportion of urine specimens contaminated by microorganism in which the test result is positive; and the specificity (Sp) as the proportion of specimens without microorganism contamination in which the test result is negative.

We also calculated the likelihood ratio (LR). A positive LR (LR+) indicates how many times a positive result is more likely to be observed in specimens contaminated by microorganisms than in those without contamination. A negative LR (LR-) indicates how many times a negative result is more likely to be observed in specimens contaminated by microorganisms than in those without contamination. The more LR differs from 1, the more accurate the test is. LR above 10 and LR below 0.1 were considered convincing diagnostic evidence (Jaeschke *et al.*, 1994).

The diagnostic odds ratio (DOR), defined as the ratio of the odds of positive test results in specimens with microorganism contamination relative to the odds of positive test results in specimens without microorganism contamination, was also calculated (Glas *et al.*, 2003). The DOR does not depend on prevalence and its value ranges from 0 to infinity, with higher values indicating better discriminatory test performance. The positive predicted value (PPV) represents the proportion of test-positive specimens that truly present the contaminated specimens, while the negative predicted value (NPV) represents the proportion of test-negative specimens that truly do not present the microorganism contaminations.

RESULTS

A total of 962 urine samples were analysed using both HB&L-Uro4 system and BiesseBioscreen. 528 samples (54.9%) were classified by BiesseBioscreen as culture negative and 208 (21.6%) as culture positive. In comparing HB&L-Uro4 and BiesseBioscreen values, each sample was classified as follows: true-positive/negative if positive/negative for both; false-positive if positive for BiesseBioscreen but negative for HB&L-Uro4; false-negative if negative for

BiesseBioscreen but positive for HB&L-Uro4.

According to the criteria described above, 224 (23.3%) specimens of the total were false positive and 2 (0.2%) false negative. The results are reported in table 1.

Furthermore the results were analysed considering separately the values obtained from the samples measured in the first and second batches (Table 2a, b). The values reported in table 2b, where the results were improved using isotonic solution, show that BiesseBioscreen can be used as a screening system, reducing the number of urine culture tests of a 61%, since culture should be performed only on the 185 BiesseBioscreen positive values.

Hence from these results we find consistency between BiesseBioscreen and culture test.

The data analysis performed to evaluate BiesseBioscreen was done calculating sensitivity, specificity, LR+, LR-, DOR, positive predicted value (PPV), negative predicted value (NPV), false negative rate (FNR), and false positive rate (FPR) according to European Committee on Antimicrobial Susceptibility Testing (www.EUCAST.org/document/sops).

Tables 3 and 4 reports these values considering

TABLE 1 - Comparison of BiesseBioscreen urine screening results according to TV standard method (HB&L-Uro4 system).

	Uro4 HB&L	
BiesseBioscreen	+	-
+	208	224
-	2	528

TABLE 2 a e TABLE 2 b - Comparison of BiesseBioscreen urine screening results according to HB&L-Uro4 results (the standard diagnosing for urine culture), for samples analyzed in the first (a) and second (b) run.

a		
	Uro4 HB&L	
BiesseBioscreen	+	-
+	108	139
-	1	233
b		
	Uro4 HB&L	
BiesseBioscreen	+	-
+	100	85
-	1	295

TABLE 3 - Performance of BiesseBioscreen method for bacteriuria screening compared to standard methods in detecting clinically relevant urine infections, considering totally and separately the samples of the first and the second runs.

	Number of samples	Sensitivity ^a (%)	Specificity ^b (%)	PPV ^c (%)	NPV ^d (%)	FNR ^e (%)	FPR ^f (%)
First run	481	99.1	62.6	43.7	99.6	0.92	37.4
Second run	481	99.0	77.6	54.0	99.7	0.99	22.4
Total samples	962	99.0	70.2	48.1	99.6	0.95	29.8

^aSensitivity = number of true positives/(number of true positives + number of false negatives). ^bSpecificity = number of true negatives/(number of false positives + number of true negatives). ^cPPV= positive predicted value. ^dNPV= negative predicted value. ^eFNR= false negative rate. ^fFPR= false positive rate.

totally and separately the first and second runs. Data analysis of BiesseBioscreen performance in bacteria screening showed very good testing results. FPR, defined as false positives over the sum of (false positive + true negative), decreased from 37.4% to 22.4% in the second experiment since the number of false positive samples was strongly reduced from 139 to 85 samples, that is less than 15.0%. This reduction can be ascribed to a more accurate protocol in view of the fact that the use of isotonic solution yields a reduction in blank counts, reflecting a more accurate measure. For the same reason the PPV, defined as the true positives over the sum of (true positive + false positive), was strongly enhanced from 43.7% to 54.0%, while NPV, defined as the true negatives over the sum of (true-negative + false-negative), results were very high in all cases. NPV was close to unity in both tests as the false negative samples were very few (2 cases) in comparison to the true negative samples (528). FNR defined as false negative over the sum of (real positive + false negative), was under 1.0 % in all cases. Sensitivity values were very close to 100%, due to only 2 false negatives, and specificity around

70% with the very high NPV indicating that the method is suitable for identifying negative samples. Analogously the LR results reported in table 4 gave values lower than 0.1 meaning a test excellence in detecting negative samples. On the other hand, the LR+ values obtained between 2 and 5 reveals a lower reliability in diagnosing positive samples, but this is in line with the target of BiesseBioscreen as a method for screening negative samples.

From these results we can conclude that BiesseBioscreen performance in bacteria screening is reliable and equivalent to standard methods.

The different results between the first and second batches can be ascribed to the different measurement protocol. Indeed in the second run, two different preparations were made for each sample to minimize the blank counts. Isotonic solution (sodium chloride 0.9%) or bidistilled water was used as blank solution and the results compared (data not shown). The isotonic solution resulted the best choice having the lower blank counts, for this reason, only the results with isotonic solution have been reported for the second run (Table 2b). These differences in protocol resulted in improved specificity in the second run, demonstrating that the isotonic solution is the best solvent to use.

TABLE 4 - Likelihood ratio considering totally and separately the samples of the first and the second runs.

	Number of samples	LR+ ^a	LR- ^b	DOR ^c
First run	481	2.652	0.0146	181.644
Second run	481	4.426	0.0128	345.781
Total samples	962	3.325	0.0136	244.485

^aLR+= positive likelihood ratio. ^bLR- = negative likelihood ratio. ^cDOR = diagnostic odd ratio.

DISCUSSION

In the past two decades several instrument-based methods have been developed for the detection and identification of microorganisms. Most have been focused primarily on automated and faster systems. However these systems are generally expensive, time-consuming and require a dedicated laboratory area

and skilled personnel. The BiesseBioscreen method would have a user-friendly interface and could be easily integrated in small laboratories, with a measuring time of less than 10 minutes, much lower than the time needed for a culture response or even for an HB&L result. In the present study the BiesseBioscreen system, compared to the standard method, demonstrated the equivalence of this system versus the traditional approach.

We compared a new way for measuring urine culture to a standard methodology already approved in microbiology. Indeed the HB&L-Uro4 system has been extensively studied in the last 20 years in several publications (Milagro A., 1999; Roveta S., *et al.*, 2004; Roveta S., *et al.*, 2006; Fontana C. *et al.*, 2009; Cermàk P. *et al.*, 2009; Barocci S. *et al.*, 2010; Fortina G., 2009; Kroumova V. *et al.*, 2010; Ilki A., 2010; Tessari *et al.*, 2010) finding the HB&L-Uro4 a reliable system for routine use in laboratories giving microbial growth results in 3 hours. As we propose BiesseBioscreen as a screening method to reduce the number of samples to be further investigated with plate culture in a microbiology laboratory, we decided to refer as standard to the HB&L-Uro4 method. Indeed for our aim, we consider HB&L-Uro4 an acceptable reference system.

The comparison between the diagnostic test and the reference standard has been represented in 2x2 contingency tables. Common indicators of test performance derived from such 2x2 tables are: the sensitivity of the test, its specificity, the positive and negative predicted values, and the positive and negative likelihood ratio (Sackett D.L. *et al.*, 1991). Overall the BiesseBioscreen system showed an excellent diagnostic accuracy, compared with the standard HB&L-Uro4 analysis, in terms of all statistical parameters. The high sensitivity and the high NPV obtained demonstrate that BiesseBioscreen analysis can be used to identify negative samples, which do not need further culture testing. In particular the 45% of positive samples obtained with this methodology implies that 530/962 (55%) samples could be excluded from urine culture after BiesseBioscreen analysis. This reduction of avoidable urine culture sample examinations could lead to a decrease of laboratory analysis costs. Furthermore the short time (less than ten

minutes) needed to identify a negative sample could save on workload and unnecessary antibiotic therapy.

Moreover, to explore the possibility to improve the statistical performance of BiesseBioscreen, we reinvestigated the false negatives belonging to the second batch of measurements. Initially the BiesseBioscreen value was 35,000 bacteria/ml while the HB&L-Uro4 result was 150,000 CFU/ml. This sample (stored at 4°C and added with boric acid as preservative) was also re-measured after 3 days with BiesseBioscreen giving 30,000 bacteria/ml, a negative value consistent with the previous measurement. Moreover for this sample the BiesseBioscreen result was also compared with the traditional quantitative culture test result performed by the microbiology laboratory to identify the isolated pathogens. The culture result was negative: 20,000 CFU/ml, a contrasting value with HB&L-Uro4 result. Nevertheless this sample was classified as positive because the microbiology laboratory of the hospital in case of contrasting results, considers the higher value more reliable. Therefore 1 of the 2 false negatives should not be considered a false but true negative. In this case, it is plausible that the HB&L-Uro4 gave a wrong result (Cermàk *et al.*, 2009).

In conclusion the BiesseBioscreen results are comparable to the standard methodology. The higher negative predicted value NPV (99.6%) and the lower false-negative rate FNR (0.95%) obtained make the method suitable as a screening system, improving the turnaround time, workload and reducing the costs of urine culture.

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