A New Screening Approach For Fast And Accurate Prediction Of Positive And Negative Urine Cultures By SediMAX Compared With The Standard Urine Culture

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Abstract. Urinary tract infections (UTI) are the most frequent bacterial infections, and the detection of infection in urine samples is expensive and time-consuming. Also, in laboratories a significant proportion of samples processed yield negative results. For this, screening methods represent an important improvement towards the final UTI diagnosis. SediMAX is an automated microscopy, easier to use in laboratories due to its basic procedure and it is widely used for urine sediment analysis. In our study, we evaluated the performance of SediMAX, applying some screening parameters, compared with the gold standard methods, urine culture, to identify all the positive cases for UTI. We analysed 1185 urine samples from our daily laboratory routine. The basis of our screening model was to establish a cut-off for bacterial count (BACT), as 300 *bacteria*/µL in order to avoid missing positive cases. However, the sensitivity and the specificity achieved were not enough to identify all UTI infection in urine samples. So, in addition to BACT we have considered other parameters, such as White Blood Cell (WBC), Red Blood Cell (RBC), Yeasts (YEST), Age and Nitrates (NIT). The second screening method reached a sensitivity of 100%, that could be reliably employed in detect of UTIs.

Keywords. Urinary Tract Infection; Urine Cultures; SediMAX; Screening Method; Bacteriuria; Automated Microscopy.

1. Introduction

It is known in literature that Urinary Tract Infections (UTI) are the most frequent infections in the community and in the hospitals [1, 2]. Urine culture is essential to confirm that the patient has UTI and to ensure that the causative agent is identified so an appropriate therapy can be started. An increase in morbidity and mortality rates could be often associated with these health care-associated infections, whereby represent important concerns at health facilities [3]. The gold standard for the diagnosis of UTIs is urine culture but is laborious and moderately expensive, with a turnaround time (TAT) of 24 to 48 h., A high level of diagnostic accuracy and rapidity of TAT should be essential in the response of urine cultures, due to some doctors prescribe empirical antibiotics to the onset of UTI-like symptoms, without waiting for the outcome of the urinary examination. Any prior prescription of empirical antibiotics may lead to the antimicrobial resistance of urine tract bacteria. Indeed, large number of urine cultures significantly increases the workload for the laboratory, even though the culture results for most specimens are negative [4]. Therefore, the development of a rapid screening method for negative urine specimens will help clinicians to prevent UTIs in a much shorter time period and avoid pointless laboratory work [5].

Despite being a massive workload in the microbiology laboratory, urine culture is still the gold standard diagnostic method for UTI. Anyway, most of the results of urine cultures are negative, at the end. For this, a trustworthy screening method could reduce unnecessary cultures and could accelerate detect of negative results. The most common cause of infection is Escherichia coli, a pathogenic microorganisms, which induce urinary tract and lower urinary tract infection, in which case it is known as a bladder infection [6], and an inflammatory response, with a clinical situation characterized by leukocyturia (WBC), erythrocyturia (RBC), yeasts (YEST) and nitrates (NIT) [7]. In laboratory routine, the most analysed samples are urine culture and, approximately,75% of them are evaluated as negative [8]. For this reason, any screening method that identifies all the infected urine samples and excludes all the negative urine samples (i.e. the samples with non-significant bacteriuria) would deserve close attention [9].

In the past few years, automated, standardized, quantitative urine analysis has been presented in clinical practice and has shown high efficiency and accuracy compared to traditional sediment analysis [10]. Urine culture is still the traditional method and it is considered, also in our work, as gold standard, although it is a hard-working and slow technique. The prolonged procedure for urine culture and all the samples which are found to be negative, should be deemed as a crucial term for laboratory costs. At the same time, automated analysis, as SediMAX, offers an impartial interpretation of results, improves accuracy and reduced TAT [11, 12]. Nowadays, there are many devices about automatization for urinary sediment analysis based on different technologies. Digital optic microscopy includes a system of cameras that photographs the sample and features an automatic recognition of elements [13].

Recently, some companies have developed several completely automated instruments examining urine sediment combining in a sole analyser both physic-chemical testing and sediment particles analysis. The use of these automatic platforms allows to reduce human error due to interobserver variability and it could improve the daily laboratory routine, optimizing workflow and maintaining accuracy and precision of the test. Moreover, when evaluating the performance of automated devices, both based on either flowcytometry or imaging techniques, the use of counting chambers should be encouraged, as it is suggested by international recommendations [14].

The aim of this work was focused mainly on evaluation of the performance of SediMAX, applying some screening parameters, compared with the gold standard methods, urine culture. The purpose was the use of automated urinalysis, as a screening tool, to predict urine cultures results, thereby identifying all the positive cases for UTI, and to reduce the culture workload in the laboratory. To validate this test, we used the predictive negative value and associated the urinalysis results of bacterial count and other patients' clinical features to urine cultures at different cut-off values of Colony-Forming Units per microlitre (CFU/mL). To our knowledge, this study identifies the independent predictive negative value among patients with presumed UTI.

2. Materials and Methods

2.1. Sample Collection

A total of 1185 anonymous fresh urine samples processed during daily routine were randomly extracted and included in the study. Urine samples from patients of a wide range of ages and different genders were analysed, according within 2-8 h from sampling by routine culture, assumed as the reference method, and by a SediMAX (77 Elektronika, distributed by Menarini Diagnostics, Italy) digital image analyser. Specimens were collected in sterile disposable tubes without preservatives, according to the European Urinalysis Guidelines, and stored and transported at ≤4 °C to Lifebrain s.r.l. for analysis. All procedures were in accordance with institutional and national ethical standards and the Declaration of Helsinki (2008).

2.2. Microbiological analysis

Urine culture is the traditional method to research pathogenic microorganisms in the urine which are possibly index of UTI. The samples were directly sown on a BD CHROMagar Orientation chromogenic medium plate (Becton Dickinson GmbH Company, Germany), which is a non-selective medium for the isolation, direct identification, differentiation and enumeration of urinary tract pathogens.

The components of CHROMagar medium include only the following: agar, peptone and yeast and "chromogenic mix." In BD CHROMagar Orientation Medium, especially selected peptones supply the nutrients. The chromogen mix releases differently coloured compounds upon degradation by specific microbial enzymes, thus assuring the direct differentiation of certain species or the detection of certain groups of organisms. The confirmatory tests were executed, according to the manufacturer's indications.

Culture was performed by inoculating 10 μL of well-mixed urine using a calibrated loop onto chromogenic agar plates, ensuring proper loading of sample down to the middle of plate. Plates were examined after incubation at 37 °C aerobically for 24 h. Once the colours of the colonies have developed, it is possible to read the plate, establishing the test result, negative or positive, based on the bacterial load (CFU for each mL of sample).

2.3. Patient Specimens and Electrophoresis Interpretation

The samples were analysed using the automatic microscopy sediment analyser SediMAX (77 Elektronika, distributed by Menarini Diagnostics, Italy) uses cuvette-based automated microscopy. This autoanalyzer homogenizes and transfers the urine samples into special disposable cuvettes, which are centrifuged for a few seconds. The sediment, generated by centrifugation, is analysed by a bright field microscope at 400× enlargement. The microscope is automated with a built-in digital camera can take up to 15 images from different zones of the cuvette with an image magnification close to 400×. The images are evaluated by a processing software which bases urine particle recognition on size, shape, and texture features. Images and results can be stored in a database and are available for re-evaluation of the samples by the operator.

The results obtained were compared with gold standard, the urine culture.

2.4. Statistical analysis

Firstly, the results of the bacterial counts (BACT) were compared and analyzed with Receiver Operating Characteristic (ROC) curve analysis. Using urine specimen culture as the gold standard, sensitivity, specificity, true positive (TP), false negative (FN), true negative (TN) and false positive (FP) rates were calculated. The predictive probability was then analysed using the ROC curve to evaluate its diagnostic ability. The best cut-off was evaluated by Youden Index.

Secondarily, the correlation of BACT to White Blood Cells (WBC), Red Blood Cells (RBC), Yeasts (YEA), Nitrates (NIT) and AGE variables were included in the evaluation using the Bayes' Theorem. These additional parameters were always compared against the gold standard method results.

3. Results

In this study, we have analysed urine samples and determined bacteria levels to establish a cut-off to may reduce the number of specimens cultured.

We have performed the urine screening of 1185 healthy non-hospitalized patients, analysed in Lifebrain s.r.l. (Rome, Guidonia; Italy) carrying out a parallel analysis with SediMAX and urine culture, the gold standard. All these urine samples were tested by using SediMAX, an automated analyser. The male percentage was 72%; the female percentage was 68%.

All urine specimens, included in this study, were tested at the same time by urine culture, the gold standard method. Statistical analysis has been performed by ROC curve analysis to determine cut-off value, diagnostic sensitivity and specificity of bacteriuria (*bacteria*/µL).

3.1. First screening method: bacterial count cut-off

The ROC curve is shown in Figure 1. It was found that the SediMAX classification as negative case was highly accurate with a diagnostic performance in terms of sensitivity and specificity of 72,60% and 92,80% respectively and with an Area Under Curve (AUC) of 89,10%. The best cut-off value obtained by means of ROC analysis was 300 *bacteria*/µL. Against this experimental cut-off value, 316 true-positive (TP) results, 54 false-positive (FP) results, 696 true-negative (TN) results, and 119 false-negative (FN) results were found and we evaluated the predictive Positive (85,4%) and Negative values (88,4%) using Bayes' Theorem in Figure 2A.

3.2. Second screening method: inclusion of other parameters

For the previous reasons, we chose to investigate more to increase the predictive Negative value. Other patient's features, as WBC or LE, RBC, YEST, NIT and AGE were considered and evaluated. The results achieved, due to the new parameters added to the initial cut-off, have showed a higher sensitivity 100%, a specificity of 90,1%, predictive Positive value of 85,5% and a predictive Negative value of 100%, as it proven in Figure 2B. Though, from Figure 2, we can observe that specificity decreased from 92,80% to 90,10%, but these data are not primarily required for our scope. In contrast, sensitivity highly increased from 72,6% to 100%.

4. Discussion

UTIs are a common infection and one of the most analysed specimens in clinical microbiological laboratories. The aim of the present study was to evaluate the prediction of positive and negative urine cultures by SediMAX compared with the gold standard method, the urine culture analysis. SediMAX is precise tool to detect the bacterial counts. Furthermore, the image quality of the urine sediment mimics traditional microscopy and none of urinary sediments are lost because they are saved in the software and can be reviewed later, in case of doubts or request by the clinician.

The basis of the our screening model was to establish a cut-off for bacterial count considering other parameters, which differs from conventional screening models based just on fixed cut-off for bacteria-counts [3, 14, 15], in order to identify all the UTIs.

In literature numerous studies report new rapid approaches for diagnosing UTIs [12]. All these tests are based on detecting the presence of bacteria and/or leukocytes in urine samples and they suggest bacteria counts of 10×10^4 CFU/mL for diagnosis of UTI [12, 15]. However, in our study we established a cut-off of 300 *bacteria*/µL for determinate positivity or negativity to the tests. In this way, differently from others, we kept a good balance between both specificity and sensitivity. This first bacterial screening generated using this analyzer, may be useful to exclude UTI and may contribute to the reduction of unnecessary urine cultures.

Although the good rate achieved from the cut-off, we wanted to deepen our study, trying to raise specificity and to decrease predictive Negative value. In this optic, more factors were contemplated and examined in order to avoid the risk of not examine urine culture which may be positive for UTI. In-depth studies have shown that possible candidates to determine better cut-off, besides bacterial counts, were presence of leucocytes, presence of erythrocytes, level of leucocyte esterase, age, gender,

presence of nitrates, presence of crystals and presence of proteins in the urine sample. Anyway, we had selected just few of the previous ones, which have proven to be sufficient. So, we had assumed new screening parameters, including BACT, WBC, RBC, YEST, AGE and NIT, to avoid all the false negative cases and to reach a sensitivity of 100%. These parameters have been useful for a more accurate discrimination between negative and positive urine samples of infection. We achieved medium levels of bacteriuria, a sharp drop in False Negative Rate, despite a slightest loss of specificity and an whole increase of the sensitivity of the SediMAX. Anyhow, each laboratory may select higher or lower cut-off values for bacterial counts if get better sensitivity is their own goal to screen UTI. Overall, the combination of these values improved the performance of the screening process and allowed a reduction of 57,95% (total of 1185 samples) in bacterial culture while avoiding level of False Negative Rate 0% (total of 1185 samples).

Furthermore, the screening by SediMAX could have an important impact in the reduction of National Healthcare System costs and in the case of our laboratory, nearly 58% of daily urine samples could be excluded from culture providing a significant reduction in workload, costs and TAT. Finally, our research achieved a predictive Positive value as 1. Our screening added to automatic analysers reduces the number of hours spent on manual review, as reported in several studies [10, 15]. In this way, SediMAX could yield a better workflow due to its precision and accuracy.

5. Conclusion

Our data showed that SediMAX plus our screening method is a precise tool, which identify all negative urine samples, allowing a better workflow. Even if the screening has a high False Positive Rate, these were correctly detected by urine cultures. Compared to the other systems the use of our screening method plus SediMAX platform offers obvious advantages.

To conclude, this study got in our laboratory an improvement of workflow efficiency and an upgrade of analytical quality. SediMAX with our screening could be used as a fast and accurate predictor of positive and negative urine infections, compared with gold standard method.

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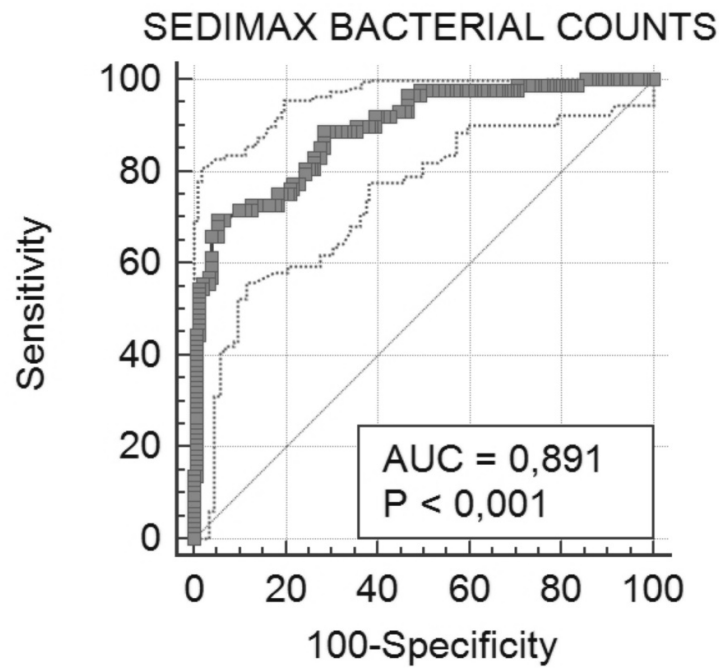
Figure 1

SEDIMAX BACTERIAL COUNTS

AUC = 0,891
P < 0,001

Figure 2

A

1st Screening Method

	Test +	Test -
UTI disease +	316	119
UTI disease -	54	696

	Test +	Test -
UTI disease +	TP	FN
UTI disease -	FP	TN

		Confidence interval at 95%	
		lower limit	upper limit
Sensitivity	0,726	0,7	0,751
Specificity	0,928	0,911	0,942
Prevalence	0,367	0,34	0,395
Positive Predictive Value	0,854	0,832	0,873
Negative Predictive Value	0,854	0,832	0,873

B

	Test +	Test -
UTI disease +	435	0
UTI disease -	74	676

	Test +	Test -
UTI disease +	TP	FN
UTI disease -	FP	TN

		Confidence interval at 95%	
		lower limit	upper limit
Sensitivity	1	0,996	1
Specificity	0,901	0,883	0,917
Prevalence	0,367	0,34	0,395
Positive Predictive Value	0,855	0,885	0,874
Negative Predictive Value	1	0,996	1

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Figure captions

Figure 1 ROC curve was obtained by using cut-off value at 300 *bacteria*/µL. The AUC evaluated was 89,0%. The Confidence Interval of 95% is between 0,845 to 0,928. The significance level p is <0,0001.

Figure 2.A) Sensitivity, specificity, positive and negative predictive values of SediMAX comparing to Urine Culture applying the cut-off based on bacterial count. **B)** Sensitivity, specificity, positive and negative predictive values of SediMAX comparing to urine culture at second screening applying BACT, WBC, RBC, YEST, AGE, NIT parameters. [UTI disease +: presence of disease; UTI disease -: absence of disease; Test +: positivity to the test; Test -: negativity to the test; TP: True Positive; FN: False Negative; FP: False Positive; TN: True Negative].