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Bacteriuria Screening by Automated Whole-Field-Image-Based Microscopy Reduces the Number of Necessary Urine Cultures

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We evaluated a new automated urine sediment analyzer that provides whole-field images for the screening of urine samples prior to bacterial culture. Sterile urine samples from 1,011 male and female outpatients and inpatients (mean age 54.7) with a urinary tract infection prevalence of 18.3% were studied. Screening rapidly provides negative results.

Urinary tract infection (UTI) is one of the most common diseases diagnosed in the clinical microbiology laboratory through bacterial count per volume of urine. Approximately 80% of urine cultures are negative (13), and screening urine samples with significant bacteriuria from those without (4, 9, 11, 13, 14) will anticipate negative results and reduce labor. In the present study, we used a new automated urine sediment analyzer that provides whole-field images of the sediment, sediMAX (Menarini Diagnostics, Florence, Italy) (16), for the screening of urine samples without significant bacteriuria.

A total of 1,011 consecutive midstream clean catch and catheter urine specimens were collected in sterile containers from both 213 inpatients and 798 outpatients, of all age groups (range, 0 to 95 years; mean, 54.7 years) and both genders, from November 2009 to January 2010, and examined within 3 h of receipt in the laboratory.

Cultures were performed by inoculating urine samples on a chromIDCPS agar (bioMérieux, Florence, Italy) (3) plate using a 1-μl loop and were incubated overnight at 37°C. Quantification in CFU/ml was done by manual count of the colonies growing on the agar plate multiplied by the dilution factor. Samples were considered positive if they contained $\geq 10^5$ CFU/ml or $\geq 10^4$ CFU/ml if they came from patients either catheterized or on antibiotic therapy. Samples with mixed cultures in which two or more organisms were isolated and with biochemical indices of infection were considered positive according to the guidelines of the Associazione di Microbiologia Clinica Italiana (AMCLI) (2) and Health Protection Agency of the United Kingdom (8).

Screening was performed with the sediMAX for automated urine sediment analysis within 15 min from culture.

A sample was evaluated as being positive for screening if the white blood cell count (WBC) exceeded 4 cells/high power field (HPF) (18 cells/μl) and/or the particle count for bacteria exceeded 10 elements/HPF (44 elements/μl); cutoff values have been established by our laboratory through a preliminary study (see the supplemental material).

Fifty-nine of the 1,011 urine samples were excluded from the final evaluation due to urine sediment particle overcrowding. Of the remaining 952 specimens, 778 (81.7%) were classified as culture negative and 174 (18.3%) as culture positive according to the criteria described above. The microorganisms isolated from the positive cultures were the following: *Escherichia coli* (n = 105; 60.3%), *Enterococcus faecalis* (n = 12; 7.0%), *Proteus mirabilis* (n = 9; 5.2%), *Klebsiella pneumoniae* (n = 9; 5.2%), *Pseudomonas aeruginosa* (n = 5; 2.9%), *Candida albicans* (n = 5; 2.9%), *Morganella morganii* (n = 3; 1.7%), *Staphylococcus epidermidis* (n = 3; 1.7%), *Citrobacter freundii* (n = 1; 0.6%), *Alcaligenes faecalis* (n = 1; 0.6%), *Staphylococcus aureus* (n = 1; 0.6%), *Providencia stuartii* (n = 1; 0.6%), and polymicrobic flora (n = 19; 11.0%). The incidence of samples classified as having significant bacteriuria by the sediMAX was 51.5% (490/952), while the incidence of those without was 48.5% (462/952).

Assessment of screening performance by sediMAX was done by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency, false-negative rate (FNR), and false-positive rate (FPR) according to Clinical and Laboratory Standards Institute (CLSI) document EP12-A (5) and was compared to that by automated strip analysis (Table 1).

Table 2 indicates the distribution of urine sediment results according to the criteria used for classifying positive samples compared to urine culture results with corresponding bacterial quantification.

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TABLE 1 Performance of urine sediment analysis compared to dipstick analysis when urine culture positivity is established at 10^4 CFU/ml

Test	Characteristics	Result (%)					
		Sensitivity	Specificity	PPV	NPV	FNR	FPR
Urine sediment	WBC > 4 cells/HPF, bacteria > 10 elements/HPF	98.3	59.0	34.9	99.4	1.7	41.0
Dipstick	Leukocyte esterase > 25 leu/μl, nitrites positive, blood > 0.03 erythrocytes/μl	33.1	98.6	82.4	88.2	66.9	1.4

TABLE 2 Comparison of urine sediment results classified according to the criteria used with urine culture results according to bacterial quantification

Sediment results			No. of samples with urine culture result[a]						
Bacteria > 10 elements/HPF (44 elements/μl)	WBC > 4 cells/HPF (18 cells/μl)	No. of samples	Negative	Polymicrobic flora ≤ 10 ^4 CFU/ml[b]	Polymicrobic flora ≥ 10 ^4 CFU/ml	≥10 ^4 CFU/ml	≥10 ^5 CFU/ml	≥10 ^6 CFU/ml	Yeast
Negative	Negative	462	459			1	2		
	Positive	52	42	1	1		4	2	2
Positive	Negative	267	169[c]	37	8	5	7	39	2
	Positive	171	66	4	10	1	18	71	1

[a] The shaded area represents the 319 false-positive samples.
[b] A count for polymicrobic flora of ≤10$\hat{4}$ CFU/ml is not clinically significant, and therefore, samples with this type of flora were considered negative by urine culture.
[c] 126 out of 169 urine sediment samples that were positive for bacteria, negative for WBC, and negative for bacterial growth when cultured had images without bacteria but only with debris when manual editing of the images was performed.

The prevalence of UTI in our study was 18.3%, similar to that obtained by other authors (Jolkonnen et al. [10], 16.8%, and Manoni et al. [12], 20.71%), and pathogen distribution was also in good agreement with data from the literature (6).

sediMAX performance in screening for significant bacteriuria (sensitivity 98.3% and NPV 99.4%) was better than strip analysis alone (sensitivity 33.1% and NPV 88.2%) (Table 1). Lammers et al. (11) reported that strip analysis was equivalent to sediment analysis. The difference in findings is probably due to the populations studied. sediMAX performance was comparable to if not better than that of other automated sediment analyzers (1, 7, 15; also see Table S3 in supplemental material) and indicate that it is suitable for screening out negative samples. Indeed, the FNR was 1.7%.

Taking a closer look at the three FN results, one can observe that the samples did not have significant bacteriuria nor WBC count (Table 2). They came from 3 outpatients, 64 years and older, with a clinical history of tumoral immunosuppression in which one would not perform a prescreening by sediment analysis but would base conclusive information regarding diagnosis of possible UTI only on urine culture results.

The resultant 41% FPR implies the unnecessary culture of slightly more than one third of our samples. However, of the total false-positive (FP) samples, 13.2% (42/319) showed polymicrobic growth (≤10 ^4 CFU/ml) when cultured (Table 2). Since this was not considered to be clinically significant, it was classified as a negative result for urine culture (Table 2). Furthermore, 267/952 urine sediments were positive for bacteria but negative for WBC counts and 169/267 did not show any growth when cultured (Table 2). Of these, 126/169 had corresponding images which showed no bacteria but only small debris which was erroneously classified as bacteria, as also seen by Zaman et al. (16). Thus, 126/267 (47%) of the samples can be excluded from urine culture by reclassification of the elements through manual editing of the whole-field images obtained with the sediMAX when the sediment shows a negative WBC count, a positive bacteria count, and no bacteria but only small debris. This would still leave an FPR of about 20%; however, this is compensated for by streamlining of the entire process. Finally, 38.6% (66/171) of the samples with positive count for both bacteria and WBCs (Table 2) had no bacterial growth when cultured, probably a consequence of ongoing antimicrobial therapy at the time of sample collection.

A cost-benefit analysis of the screening performed on 952 samples by dipstick showed a cost of about 315 euros (0.33 euros/sample), and although this is relatively inexpensive, it is not acceptable in our case due to the high number of false-negative results obtained by this method (see Table 1). The same screening performed only with the sediMAX costs about 400 euros (0.42 euros/sample) plus 76 euros for expert staff time for manual editing of 126 samples. Screening for significant bacteriuria with sediMAX would lead us to save a total of 699 euros (1.2 euros/sample) on the bacterial cultures performed. Therefore, the new method delivers better results at a slightly reduced cost (476 versus 699 euros). However, the most important saving obtained with this method is the time needed for a negative result, from 24 h to minutes, and thus, savings on needless antibiotic therapy and hospital stay.

In conclusion, our data show that the sediMAX is a useful tool for screening urine samples. It rapidly excludes those that are negative for significant bacteriuria from further processing by culturing, allowing physicians to promptly make clinical decisions upon receipt of negative results. Additionally, this also reduces the number of unnecessary urine cultures performed, easing both costs and workload.

REFERENCES

1. **Accordini A, et al.** 2007. Microscopia automatizzata e screening delle batteriurie. 21° Congresso Nazionale Società Italiana di Medicina di Laboratorio (SIMeL). Società Italiana di Medicina di Laboratorio, Venice, Italy. http://www.il-italia.it/documenti/letteratura/flyer_Accordini[1].pdf.
2. **AMCLI.** 2008. Proposta di Percorso Diagnostico IVU. XXXVII Congresso Nazionale Associazione di Microbiologia Clinica Italiana (AMCLI), Stresa, Italy, 5-7 October 2008.
3. **bioMérieux.** 2009. Chromogenic medium for the enumeration of organisms in urine samples and the direct identification of *Escherichia coli*, *Enterococcus*, KESC and *Proteae*. bioMérieux, Marcy-l'Etoile, France.
4. **Bowman RA, Riley TV.** 1991. Evaluation of Clinitek 200 and RapimatII/T for screening for urinary tract infection. J. Clin. Pathol. **44**:58–60.
5. **CLSI.** 2007. User protocol for evaluation of qualitative test performance; approved guideline. CLSI document EP12-A2. CLSI, Wayne, PA.
6. **Foxman B.** 2010. The epidemiology of urinary tract infection. Nat. Rev. Urol. **7**:653–660.
7. **Gessoni G, et al.** 2004. Diagnosis of acute urinary tract infections using Sysmex UF-100. Sysmex J. Int. **14**:18–22.
8. **Health Protection Agency.** 2009. Investigation of urine. BSOP 41. Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London United Kingdom.
9. **Hughes C, Roebuck MJ.** 2003. Evaluation of the Iris 939 UDx flow microscope as a screening system for urinary tract infection. J. Clin. Pathol. **56**:844–849.
10. **Jolkkonen S, Paattiniemi EL, Karpanoja P, Sarkkinen H.** 2010. Screening of urine samples by flow cytometry reduces the need for culture. J. Clin. Microbiol. **48**:3117–3121.
11. **Lammers RL, et al.** 2001. Comparison of test characteristics of urine dipstick and urinalysis at various test cutoff points. Ann. Emerg. Med. **38**:505–512.
12. **Manoni F, et al.** 2002. Field evaluation of a second-generation cytometer

UF-100 in diagnosis of acute urinary tract infections in adult patients. Clin. Microbiol. Infect. **7**:662–668.
13. **Okada H, et al.** 2000. Detection of significant bacteriuria by automated urinalysis using flow cytometry. J. Clin. Microbiol. **38**:2870–2882.
14. **Palmer LS, Richards I, Kaplan W.** 1997. Clinical evaluation of a rapid diagnostic screen (Uriscreen) for bacteriuria in children. J. Urol. **157**:654–657.
15. **Pieretti B, et al.** 2010. Diagnosis of bacteriuria and leukocyturia by automated flow cytometry compared with urine culture. J. Clin. Microbiol. **48**:3990–3996.
16. **Zaman Z, et al.** 2010. Urine sediment analysis: analytical and diagnostic performance of sediMAX®—a new automated microscopy image-based urine sediment analyser. Clin. Chim Acta **411**:147–154.