Dolunay Gülmez* and Gülşen Hasçelik

Efficient use of laboratory resources: pre-screening for urine cultures by automated urinalysis and microscopy to allow exclusion of specimens from culture workflow

DOI 10.1515/labmed-2015-0069
Received July 6, 2015; accepted February 4, 2016; previously published online March 11, 2016

Abstract

Background: Most of the urine cultures give negative results, leading to inefficient use of resources. The aim of this study was to evaluate and optimize screening by urinalysis and urine microscopy allowing elimination from the culture workflow, and find out if it might be cost effective in Turkey.
Methods: A total of 1511 urine specimens were evaluated. Urinalysis and automated microscopy was performed by Iris iQ200 (Iris Diagnostics, USA). Results for nitrite, leukocyte esterase (LE), white blood cell (WBC), red blood cell (RBC) and all small particles (ASP) were compared to urine culture results. Savings were calculated, in case specimens predicted to be negative were not cultured.
Results: Microbial growth was detected in 279 (18.5%) specimens. Using the most efficient algorithm, 400 (26.5%) specimens could be excluded from the workflow, leading to three (0.2%) false negatives. Second algorithm could predict negative result for 15.7% of the specimens with a negative predictive value (NPV) of 100%, saving $562.
Conclusions: Screening urine specimens using multiple criteria might help predicting urine culture results. Although the cost of urine culture is low in Turkey, screening might still decrease cost and workload. All the variables should be considered to achieve efficient management of resources in the healthcare system without compromising patient safety.

***Correspondence: Dolunay Gülmez,** Hacettepe University Faculty of Medicine, Medical Microbiology Department, Ankara, Turkey, Tel.: +90 312 3051560, E-mail: dolunayglm@gmail.com; dolunay@hacettepe.edu.tr
Gülşen Hasçelik: Hacettepe University Faculty of Medicine, Medical Microbiology Department, Ankara, Turkey

Keywords: cost analysis; urinalysis; urine culture screening.

Introduction

Urinary tract infection (UTI) is a common health problem in both outpatient and inpatient settings. Although most of the community-acquired UTIs are diagnosed according to clinical signs and symptoms, urine cultures are still needed to detect the pathogen and its antimicrobial susceptibility in case of treatment failure in 48 h and in complicated infections [1, 2]. Besides, other diseases of the urinary tract may cause similar symptoms, leaving physicians in need for laboratory confirmation [3, 4].

Although quantitative urine culture is the gold standard for diagnosis of UTIs and urine cultures make up the majority of microbiology laboratory workload, up to 80% of these cultures can be negative [2, 3]. Therefore, screening tests that use microscopic and biochemical characteristics of urine are developed to predict the urinary infection in some patient populations [1, 3, 5]. Gram stain has an advantage of being available in every microbiology laboratory. However, it is labor intensive, may not be cost effective and sensitivity may be low for bacteriuria below 10^4 cfu/mL [3]. Nitrite positivity is associated with infections caused by nitrate reducing bacteria, and sensitivity is better in first morning specimens because bacteria need time to convert nitrate to nitrite [2, 3]. Detection of white blood cells (WBC) by microscopy may be problematic because of their quick deterioration in urine and counting leukocytes by a hemocytometer is not suitable during routine workflow [3]. Searching for leukocyte esterase (LE) might be another option, but its sensitivity is low [1]. Each test has its own advantages and disadvantages and sensitivity differs according to the method used and may be useful in patients with atypical clinical presentations [1, 3]. Individually, one test may not be enough to predict or exclude urinary infection; however, an algorithm using

multiple tests may be more successful for ruling out bacteriuria [3, 6, 7]. Urinalysis with or without urine microscopy is used frequently for screening UTIs and may help efficient use of labor, time and financial resources in the microbiology laboratory.

Screening urine using urinalysis may be used in two ways to predict urine culture outcomes. First, to detect and eliminate negative cultures, saving time and resources. Second, to separate specimens of infected patients and set up a urine culture that enables to detect the pathogen. Because not all urinary infections do need urine culture for diagnosis and treatment, the second approach may lead to unnecessary cultures [2, 4]. In addition, patient characteristics such as age, sex and underlying conditions should be considered and clearly defined cutoffs should be established in order to triage specimens for culture using the second approach [1]. Because routine workup in our laboratory lacks clinical data, this study was based on the first approach.

The aim of this study was to evaluate and optimize screening by urinalysis and urine microscopy by an automated system, compare with urine cultures and find out if specimens might be eliminated from the culture workflow and the practice might be cost effective in our hospital.

Materials and methods

A total of 1511 urine specimens submitted to microbiology laboratory of Hacettepe University Adult Hospital both for culture and urinalysis were included the study.

Automated microscopy was performed using Iris iQ200 (Iris Diagnostics, USA). For microscopy, the system uses a microscope coupled to a CCD (charge coupling device) video camera to detect particles in the specimen sandwiched between enveloping layers of suspending fluid. This allows orthoscopic particle orientation and focus the objective lens of the microscope. Five hundred fields are captured by the video camera. The Auto-Particle Recognition (APR™) software uses size, shape, contrast and texture to identify each image. Particles smaller than 3 μm were classified as "all small particles" (ASP) by the system and accepted as bacteria as recommended by the manufacturer. Concentration of particles were calculated using the number of images and the volume scanned. Reports were finalized after being reviewed by an operator. Nitrite and LE was detected by the connected iChemVelocity system (Iris Diagnostics, USA) using test strips read by wavelength reflectance and specific gravity using the refractive index [8]. Results for nitrite, LE, WBC, red blood cell (RBC), and ASP were noted.

Urine cultures were done quantitatively. Ten microliters of urine specimens were plated on blood and MacConkey agars. After overnight incubation of plates at 36±2 °C, growth of ≤2 microorganisms, each ≥10,000 cfu/mL was considered as positive [2]. Bacteria were identified by Phoenix automated system (Becton Dickinson, USA) supported by conventional tests when needed. Receiver Operating Characteristic (ROC) curves, specificity, sensitivity, negative (NPV) and positive predictive values (PPV) were calculated for all criteria with different threshold values. All statistical analysis was carried out using SPSS Ver. 18 (SPSS Inc., Chicago, IL, USA) [9]. Different thresholds were investigated in order to obtain a NPV of 100%. Savings were calculated, in case specimens predicted to be negative were not cultured. Cost of one negative culture included the media, energy and workload needed. Since the specimens were submitted for both urine culture and urinalysis independently, cost of urinalysis was not included in the calculation of savings. The cost of reportable urinalysis test in our hospital, which includes system rental and consumables, was given seperately in order to enable comparison.

Ethical approval was not required for this study.

Results

Among 1511 urine specimens, microbial growth was detected in 279 (18.5%), most of which were gram negative bacteria (78.6%). Fifty eight percent of all the positive cultures yielded *Escherichia coli*. Microorganisms detected in culture positive specimens were given at Table 1.

A total of 1232 urine specimens yielded negative culture results. Cost of one negative urine culture was $2.37 in our country, thus the financial burden of 1232 negative cultures was $2920. Cost of reportable test for urinalysis was $1, therefore cost of urinalysis for all specimens were $1511.

Specificity, sensitivity, NPV and PPV for ASP, LE, nitrite, RBC and WBC were summarized in Table 2. When nitrite, LE, WBC or RBC was used as the only criterion; a NPV of 100% could not be achieved. For a single criterion, NPV was 100% only when ASP was ≤421. If this single criterion was applied, negative results could be given for 130 (8.6%) of the specimens without culture and $308 could be saved with this analysis.

All the criteria (nitrite, LE, WBC, RBC and ASP) were analyzed to achieve the most efficient outcome or a NPV of 100%. Evaluation using nitrite, WBC and ASP was found to be significant. Receiver operating characteristic (ROC) curves of the criteria are shown in Figure 1.

The algorithm to obtain the most efficient outcome was shown in Figure 2. If this algorithm was used, a total of 400 (26.5%) specimens with nitrite negative, WBC negative and ASP<2022 results would be excluded from culture workflow. In this situation $948 could have been saved. However, three (0.2%) of these specimens had positive culture results; therefore would be a false negative. All three specimens yielded $>10^5$ cfu/mL of bacteria (*Enterobacter aerogenes*, *Enterococcus faecalis and Streptococcus agalactiae*) from outpatient female patients.

Table 1: Microbial growth results of positive urine cultures.

Microorganism	n (%)
Gram negative	221 (78.6)
Escherichia coli	163 (58.0)
Klebsiella spp.	25 (8.9)
Klebsiella pneumonia (n=15)	
Klebsiella oxytoca (n=10)	
Enterobacter spp.	4 (1.4)
Enterobacter cloacaea (n=2)	
Enterobacter aerogenes (n=2)	
Proteus mirabilis	3 (1.1)
Other Enterobacteriaceae	10 (3.6)
Morganella morganii (n=5)	
Citrobacter koseri (n=2)	
Citrobacter freundii (n=1)	
Serratia marcescens (n=1)	
Kluyvera ascorbata (n=1)	
Pseudomonas aeruginosa	8 (2.8)
Others	8 (2.8)
Acinetobacter baumanni (n=3)	
Alcaligenes faecalis (n=2)	
Achromobacter spp. (n=1)	
Pseudomonas spp. (n=1)	
Aeromonas hidrophila (n=1)	
Gram positive	54 (19.3)
Enterococcus spp.	21 (7.5)
Streptococcus spp.	19 (6.8)
Streptococcus agalactiae (n=16)	
Streptococcus mitis (n=1)	
Streptococcus oralis (n=1)	
Streptococcus pyogenes (n=1)	
Staphylococcus spp.	14 (5.0)
Staphylococcus epidermidis (n=6)	
Staphylococcus haemolyticus (n=5)	
Staphylococcus hominis (n=2)	
Staphylococcus aureus (n=1)	
Yeasts	6 (2.1)
Total	281[a] (100.0)

[a]In two of the cultures, both *E.coli* and *K.oxytoca* were obtained.

If all the criteria were evaluated to obtain 100% NPV, best results were obtained when nitrite was negative, WBC was ≤1 and ASP was ≤729 (Figure 3). In this case negative culture result could be predicted for 237 (15.7%) of the specimens and, $562 could have been saved.

Discussion

Quantitative urine culture is the gold standard for diagnosis of UTIs and, it is necessary to detect the pathogen and antimicrobial susceptibility. However, unnecessary cultures are still ordered for patients and laboratory rejection criteria cannot cover these specimens because of insufficient clinical information [1]. Screening may provide an opportunity to decrease laboratory workload by eliminating urinary infection in selected patients [1, 3]. It may also help to save costs and acquire results in a shorter time. This study focused on the practical use of screening urine samples for infection in our hospital.

Most of the urine culture orders submitted to the routine clinical microbiology laboratories do not yield significant microbial growth [2, 3]. Although culture negative results might be as low as 30% in female patients with clinical symptoms, culture might not be indicated and ampirical therapy might be appropriate for this group [10]. In this study, 81.5% of the cultures were reported as negative.

Enterobacteriaceae, especially *Escherichia coli* are the most common uropathogens isolated from urine cultures [4, 10]. In this study, *E.coli* was isolated from 58% of positive cultures and Enterobacteriaceae made up to 73% of the positive cultures. The most common gram positive bacteria were enterococci. Our results are consistent with the literature [4, 10].

Table 2: Sensitivity, specifity, PPV and NPV for selected single criteria for elimination of negative urine culture results.

	Criterion	Sensitivity	Specificity	PPV	NPV
All small particles (ASP)	>421	100.00	10.47	20.2	100.0
All small particles (ASP)	>727	97.85	23.21	22.4	97.9
All small particles (ASP)	>2022	79.57	56.57	29.3	92.4
Leukocyte esterase	>0	68.10	75.95	39.1	91.3
Leukocyte esterase	>25	56.27	86.11	47.9	89.7
Nitrite	Positive	41.22	98.05	82.7	88.0
Red blood cell	>0	46.21	54.06	18.4	81.7
Red blood cell	>10	18.77	89.12	28.0	83.0
White blood cell	>0	91.76	48.86	28.9	96.3
White blood cell	>3	74.19	79.87	45.5	93.2
White blood cell	>5	67.38	83.69	48.3	91.9

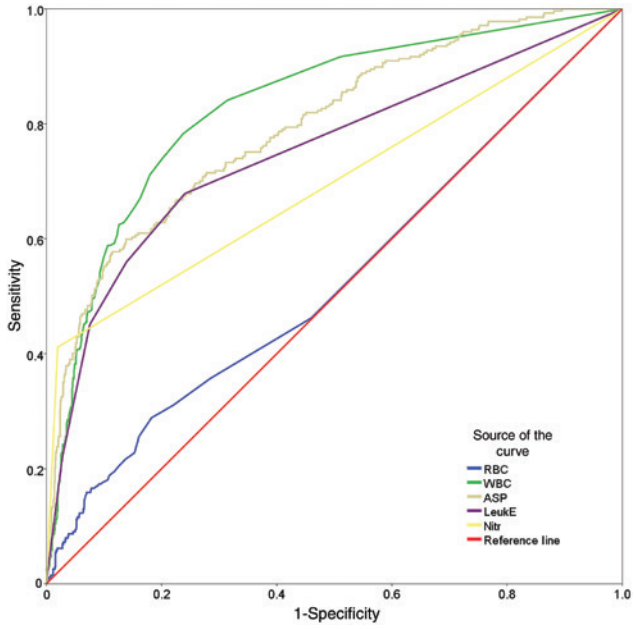

Figure 1: ROC curves for red blood cells (RBC), white blood cells (WBC), all small particles (ASP), leukocyte esterase (LeokE) and nitrite (Nitr).

Different criteria were proposed to detect urine culture outcomes and to predict urinary infection. Sterile pyuria may be present in trauma, catheterization and, some systemic and local infections therefore WBC count is nonspecific [2, 4]. In our study, even with a high threshold of >5 WBC/HPF, specificity was 83.69% (Table 2). LE is also not specific and less sensitive than WBC count [2, 4]. Table 2 shows a sensitivity as low as 56.27% with a threshold of >25 leu/μL for the specimens included in our study. Hematuria and proteinuria are common, but they are neither sensitive nor specific for detecting urinary infection [2, 4]. Hematuria of >10 RBC/HPF yielded a NPV of only 83% (Table 2) in the present study. Wong et al. used nitrite test and presence of urinary infection symptoms to eliminate infection and could exclude 29% of urine culture workup [11]. Nitrite test in urine has a low false positivity rate but may be false negative when colony counts are low and infection is not caused by nitrate reducting microorganisms [2, 4]. Our results indicated a high specificity of 98.05% with nitrite positivity. However, sensitivity was only 41.22% (Table 2). None of the tests except ASP could obtain a NPV of 100% when used as a single criterion. Therefore combined use of different tests were investigated to predict urine culture results as recommended by the literature [3, 6, 7].

Yıldırım et al. investigated the validity of Gram staining, manual WBC count, nitrite and LE dipstick tests versus quantitative culture in urine specimens of infected and uninfected patients. Although Gram staining had a high specificity (100%) and NPV (88.3%), they pointed out that dipstick tests were more practical [12]. Automated urinalysis systems that are able to perform both biochemical testing and microscopy are available and results are comparable with manual staining methods [13, 14]. Gram staining, which would increase workload, was not preferred and data provided by the automated urinalysis and microscopy system were used in this study to achieve better results.

Multiple studies showed that an ideal screening test, which has a NPV of 100% might be possible using multiple criteria. Stoval et al. could rule out infection in trauma

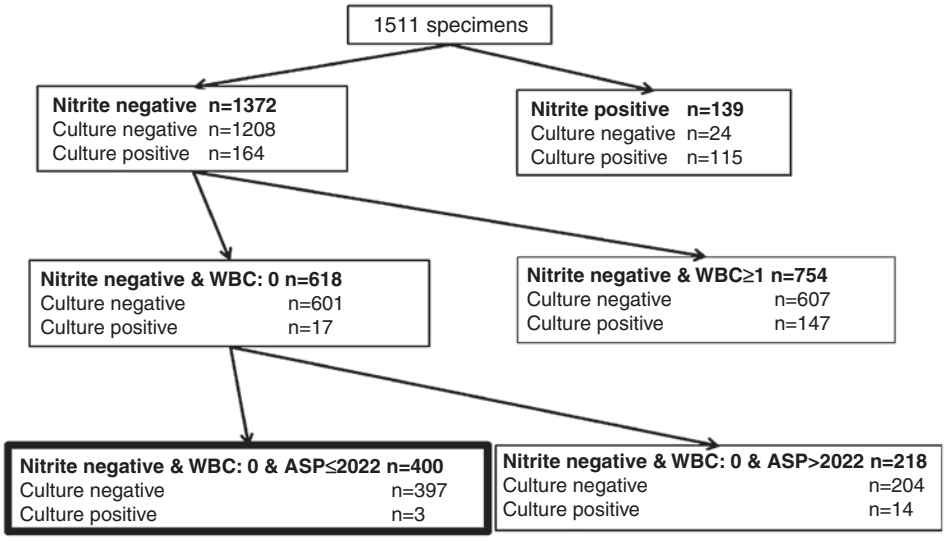

Figure 2: Most efficient criteria for elimination of negative urine culture results.

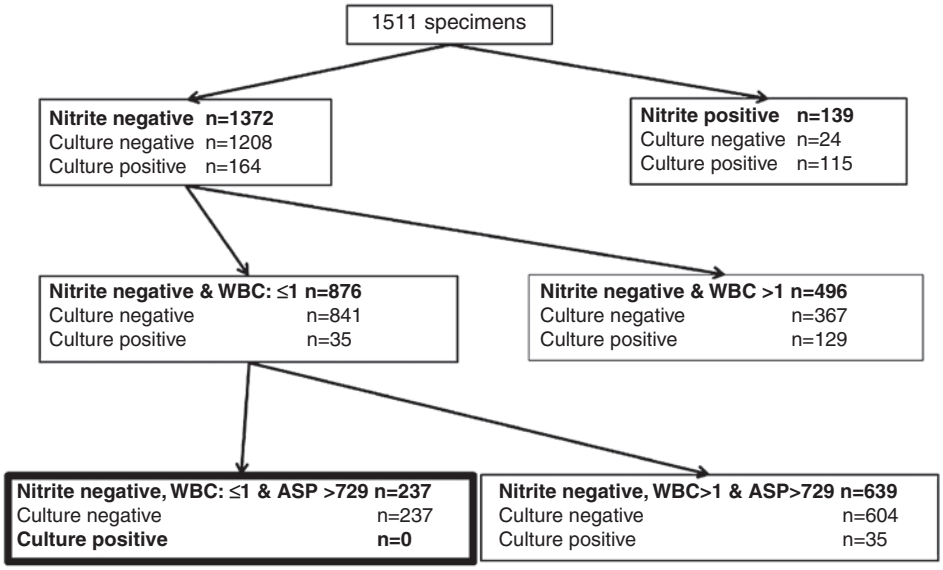

Figure 3: Criteria selected for elimination of negative urine culture results when NPV was 100%.

patients with a NPV of 100% using urinalysis to detect bacteriuria, pyuria, LE and nitrite [14]. In this study, it was possible to achieve a NPV of 100% by using multiple criteria i.e. nitrite, WBC and ASP, eliminating 15.7% of the culture workup (Figure 3).

Karakukcu et al. used bacterial counts and/or WBCs obtained from an automated system to screen urinary infections in outpatients and established cut-off values to increase sensitivity and NPV to 99.8% and 100%, respectively. However, specificity decreased to 52.0% [15]. In our study, most efficient criteria yielded a sensitivity of 98.9% and a specificity of 32.2% by using nitrite, WBC and ASP results. Lower specificity might be a consequence of the different patient population, which was not limited to outpatients and included more complicated cases from a tertiary hospital.

Kayalp et al. investigated 32,998 clean-catch urine specimens, noted that 97.7% of urine culture requests were negative and evaluated nitrite, LE, WBC and bacteria detection by an automated system [16]. Bacteriuria could detect negative cultures with a NPV of 99.5% and the PPV was highest (79.4%) when bacteriuria and nitrite combination was used. Although more specimens were evaluated, >90% of them were obtained from outpatients and emergency department which reflects a more homogenous patient profile and is the possible reason for high culture negativity. In addition, due to retrospective nature of the study, there are no data on the cases with false negative screening results. In our study, when the most efficient criteria were used (Figure 2), NPV was 99.2% but PPV was as low as 24.8%. Similar to the previous study by Karakukcu et al., this might also be caused by the different patient profile of our hospital which mostly functions as a reference center for complicated cases. In addition, three patients with negative screening had positive culture results using the most efficient algorithm therefore screening results were false negative. These were female outpatients and clinical evaluation might be appropriate to decide if urine culture was indicated.

As well as avoiding time consuming and labor intensive practices, saving costs is also an important and preferred issue in the laboratory [17]. Cost-effectiveness might change according to a hospital's patient population and the cost of a single culture and urinalysis might differ in different settings. Because this study included specimens submitted to both urinalysis and urine culture, savings were calculated according to urinalysis results that could allow elimination from the culture workflow. Using multiple criteria, an algorithm that provides NPV of 100% or most efficient outcome could predict 237 and 397 of the negative cultures, respectively, and could save $2.37 per culture.

Cost of negative cultures might increase in different settings. In our calculation, only media, energy and workload were included. Any efforts to identify the bacteria were excluded and average wage of microbiology technicians was low in our country, which decreased the cost of workload. However, Downs reported that cost of a urine culture was $21.53 (charge, $26.00) in an American hospital 15 years ago [18]. Cost of a urine culture might be as high as $88 in polymicrobial cultures [19]. Therefore cost

effectiveness should be calculated seperately for each country according to financial data.

Screening of urine specimens using multiple criteria might help clinicians and the laboratory to avoid unnecessary urine cultures and accelerate test results and patient care. Decreasing the number of unnecessary urine cultures might allow laboratories to use their limited resources more effectively. As a result of this study, it is concluded that our hospital might benefit from screening urine specimens which may allow faster results in addition to decrease of culture workload and costs. However, false negative results might occur when the most effective criteria are used and clinical information should be used to avoid compromising patient safety.

Acknowledgments: The authors would like to thank Jale Karakaya and Ergun Karaağaoğlu for statistical consulting.
Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Burd EM, Kehl KS. A critical appraisal of the role of the clinical microbiology laboratory in the diagnosis of urinary tract infections. J Clin Microbiol 2011;49:S34–8.
2. Sharp SE, editor. McCarter YS, Burd EM, Hall SG, Zervos M. CUMITECH 2C Laboratory diagnosis of urinary tract infections. CUMITECH (Cumulative Tecniques and Procedures in Clinical Microbiology). Washington, DC: ASM Press, 2009.
3. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis 2004;38:1150–8.
4. Mandell GL, Bennett JE, Dolin R, editors. Mandel, Douglas and Bennett's principles and practices in infectious diseases, 7th ed. Philadelphia: Churchil and Livingstone Elsevier, 2010:4028.
5. Fok C, Fitzgerald MP, Turk T, Mueller E, Dalaza L, Schreckenberger P. Reflex testing of male urine specimens misses few positive cultures may reduce unnecessary testing of normal specimens. Urology 2010;75:74–6.
6. Semeniuk H, Church D. Evaluation of the leukocyte esterase and nitrite urine dipstick screening tests for detection of bacteriuria in women with suspected uncomplicated urinary tract infections. J Clin Microbiol 1999;37:3051–2.
7. Cantey JB, Gaviria-Agudelo C, McElvania TeKippe E, Doern CD. Lack of clinical utility of urine gram stain for suspected urinary tract infection in pediatric patients. J Clin Microbiol 2015;53:1282–5.
8. Chapter 2: System Description. In Iris iQ200 Series Operators Manual, Rev B. Chatsworth, CA: Iris Diagnostics, 2010.
9. Zhou X, Obuchowski NA, McClish DK. Statistical methods in diagnostic medicine, 1st ed. New York: Wiley Inc., 2002:419.
10. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrob Chemother 2003;51:69–76.
11. Wong HF, Lee LC, Han HC. Cost-effective screening for urinary tract infections in urogynaecological patients. Int Urogynecol J Pelvic Floor Dysfunct 2008;19:671–6.
12. Yıldırım M, Şahin İ, Küçükbayrak A, Öksüz Ş, Acar S, Yavuz MT. The validity of the rapidly diagnostic tests for early detection of urinary tract infection. Düzce Tıp Fak Derg 2008;3:39–42.
13. Zaman Z, Fogazzi GB, Garigali G, Croci MD, Bayer G, Kranicz T. Urine sediment analysis: Analytical and diagnostic performance of sediMAX – a new automated microscopy image-based urine sediment analyser. Clin Chim Acta 2010;411: 147–54.
14. Stovall RT, Haenal JB, Jenkins TC, Jurkovich GJ, Pieracci FM, Biffl WL, et al. A negative urinalysis rules out catheter-associated urinary tract infection in trauma patients in the intensive care unit. J Am Coll Surg 2013;217:162–6.
15. Karakukcu C, Kayman T, Ozturk A, Torun YA. Analytic performance of bacteriuria and leukocyturia obtained by UriSed in culture positive urinary tract infections. Clin Lab 2012;58:107–11.
16. Kayalp D, Dogan K, Ceylan G, Senes M, Yucel D. Can routine automated urinalysis reduce culture requests? Clin Biochem 2013;46:1285–9.
17. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: a meta-analysis. Pediatrics 1999;104:e54.
18. Downs SM. Technical report: urinary tract infections in febrile infants and young children. The Urinary Tract Subcommittee of the American Academy of Pediatrics Committee on Quality Improvement. Pediatrics 1999;103:e54.
19. Darouiche RO, Priebe M, Clarridge JE. Limited vs full microbiological investigation for the management of symptomatic polymicrobial urinary tract infection in adult spinal cord-injured patients. Spinal Cord 1997;35:534–9.

Article note: This study was presented at the American Society for Microbiology 112th General Meeting. 16–19 June 2012, San Fransisco, CA.