Evaluation of Two Automated Systems for Detection of Bacteriuria

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The Utiscreen-CORAL Biomedical system and ROBOBACT system were tested against conventional uroculture in blood agar and MacConkey agar as a reference method to determine the bacteriuria from 400 samples. For the Utiscreen-CORAL Biomedical system, a sensitivity of 92.5% was obtained. However, by the ROBO-BACT system, the sensitivity was 69.9%. J. Clin. Lab. Anal. 20:118–120, 2006. © 2006 Wiley-Liss, Inc.

Key words: bacteriuria; urine; automated system

INTRODUCTION

Urine is by far the most frequent sample sent to clinical microbiology laboratories for microbial count and studies of sensitivity to antibiotics in clinically relevant cases (1). The most widely used culture media for their processing are blood agar, MacConkey agar (bioMérieux, Marcy-l'Etoile, France), and cysteine lactose electrolyte-deficient (CLED) agar. Blood agar is adequate to isolate and count bacteria in urine but is not adequate to differentiate among microorganisms, and it fails when there is growth of invasive *Proteus* species. MacConkey agar is a selective medium and CLED agar (Francisco Soria Melguizo, Madrid, Spain) prevents invasion by *Proteus* spp., but they have a low differential capacity, only allowing differentiation between lactose-fermenting and non–lactose-fermenting colonies (2), and microorganisms such as *Streptococcus agalactiae* or *Corynebacterium urealyticum* would be inhibited by CLED agar.

When a large number of samples must be processed, a bioluminescent automated system can be used to detect bacterial adenosine triphosphate (ATP), offering real-time performance of clinically relevant bacterial counts. The Utiscreen-CORAL Biomedical system (Francisco Soria Melguizo, Madrid, Spain) (3) is one such system, but although it provides indirect information on the amount of microorganisms present, it does not allow isolation of urinary tract infection (UTI)-producing agents, which must be done using other standardized procedures.

Chromogenic culture methods are also available for microbial isolation and count and for the presumptive identification of colonies, with no need for additional biochemical tests. Among these methods, an automated system has been developed (ROBOBACT; Diesse Diagnostica Senese S.p.A., La Tognazza, Italy) (4,5) that uses nonselective (CLED) and chromogenic media for the bacterial count and identification of Gram-negative and enterococcal bacteria. The system has a robotic arm connected to a 1-μL calibrated device for automatic and homogeneous seeding of the urine sample and incorporates an incubator for the incubation of cultures after their inoculation. Because of the low diversity of the microbes responsible for UTI (6), this method may be useful in clinical laboratories because it simultaneously yields the count and identification of isolates.

No study has been published on the use of the ROBOBACT system (Medline 1987–2005) for bacterial count in clinical urine samples. Therefore, our group designed a study to determine the behavior of this system and the bioluminescent Utiscreen-CORAL Biomedical system, comparing them with conventional uroculture in blood agar and MacConkey agar as reference method.

MATERIALS AND METHODS

We studied 400 urine samples received by the Microbiology Department of the San Cecilio University

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Hospital, a reference hospital center serving 310,000 inhabitants and two primary care districts in the southern Spanish province of Granada. All samples were seeded (10 µL) on plates of sheep-blood Columbia agar (bioMérieux, France) and centrifuged at $900\,g$ for 5 min; the sediment was visualized ($400\times$; cutoff point for significant leukocyturia: 4–5 leukocytes/field), and was then seeded in MacConkey agar. After a 24-hr incubation at 37°C, counts of $\geq 10^4$ colony-forming units (CFU)/mL were considered significant and those of $< 10^4$ CFU/mL negative. Urine was reported to the clinician as contaminated and a new sample was requested if, after the incubation period, more than one bacterial species grew in absence of leukocyturia in a patient who was not immunodepressed, pregnant, elderly, had an indwelling bladder catheter, or was less than 3 years old. In these cases, the sample was only considered contaminated when more than two bacteria species were present. Presence of *Staphylococcus aureus*, *Corynebacterium urealyticum*, and *Candida* spp. was assessed in all cases. The method for identifying microorganisms was previously published (6).

Each sample was also processed using the automated Utiscreen-CORAL Biomedical system, whose technical characteristics have been described elsewhere (3). If the light emission exceeded the threshold value of 2% of calibrator emission recommended by the manufacturer, the sample was considered to have a microbial count of $\geq 10^4$ CFU/mL.

At the same time, all samples were processed by the ROBOBACT system. After incubation within the system at 37°C for 24 hr, the solid media were read by a investigator, considering counts of $\geq 10^4$ CFU/mL as significant. Manufacturer's instructions were followed to identify isolates according to the color, shape, and size of colonies.

RESULTS AND DISCUSION

The results obtained are listed in Table 1. The count was $\geq 10^4$ CFU/mL in 199 (50%) of the 400 samples studied by the reference method (conventional uroculture), of which 49 were evaluated as contaminated. Utiscreen-CORAL Biomedical results showed 216 samples with a count of $< 10^4$ CFU/mL; 15 of these were significant by the reference method (sensitivity: 92.5%), of which 12 were evaluated as contaminated and three presented a single microorganism (two isolates of *E. coli* and 1 of *E. faecalis*). Sensitivity value obtained was somewhat better than the reported by Semeniuk et al. (3) (sensitivity: 86%) for threshold value of 4% of calibrator emission.

Sixty samples that were negative by ROBOBACT were found to be significant by the reference method (sensitivity: 69.9%), of which 49 were evaluated as contaminated and 11 corresponded to isolates of *Candida glabrata* (1), *Candida tropicalis* (1), *Candida albicans* (1), coagulase-negative *Staphylococcus* (4), *Staphylococcus aureus* (1), *Streptococcus agalactiae* (1), *Corynebacterium* spp. (1), and *Proteus mirabilis* (1), respectively.

According to this study, the Utiscreen-CORAL Biomedical system showed an acceptable behavior to detect low microbial counts (92.5%), with the possibility of achieving 100% sensitivity by testing more urine samples in cases of persistent clinical suspicion. For its part, the automated ROBOBACT system has several valuable features, including: availability of microorganism count and identification within 24 hr; automatic and therefore standardized seeding of samples, reducing the laboratory workload and improving the objectivity of observations (4); and the lesser handling of samples, reducing the contamination risk. However, the ROBOBACT system showed some drawbacks: yeast is not detected (which can be overcome by incorporating a Sabouraud plate in the seeding); good isolation is not always obtained when there is more than one microorganism; the assessment of a urine sample as contaminated contains a major subjective element; and, unlike in the identification, time cannot be saved in the antibiogram. However, the most important shortcoming of the system is its low sensitivity in the bacterial count, compromising the detection of contaminated urine and, more importantly, true urinary tract infections.

TABLE 1. Comparison of Two Automated Systems with the Reference Method

		Uroculture (blood and MacConkey agars)	
		$\geq 10^4$ CFU/mL	$< 10^4$ CFU/mL
Utiscreen-CORAL Biomedical®	$\geq 10^4$ CFU/mL	184	0
	$< 10^4$ CFU/mL	15	201
ROBOBACT®	$\geq 10^4$ CFU/mL	139	0
	$< 10^4$ CFU/mL	60	201

REFERENCES

1. Gutiérrez J, Rodríguez M, Morales P, Piédrola G. Importancia del riesgo de contaminación de las muestras de orinas para el diagnóstico de infección del tracto urinario en un hospital de tercer nivel. Rev Invest Clin 2000;52:581–583.
2. Palacios E, Rodríguez-Granjer J, Sanpedro A, Martínez-Brocal A, de la Rosa-Fraile M. Use of MPO chromogenic culture medium for routine processing of urine cultures. Enferm Infecc Microbiol Clin 2002;20:388–390.
3. Semeniuk H, Noonan J, Gill H, Church D. Evaluation of the Coral UTI Screen system for rapid automated screening of significant

bacteriuria in a regional centralized laboratory. Diagn Microbiol Infect Dis 2002;44:7–10.

4. Alonso-Sanz M, Molina L. Evaluation of the ROBOBACT system for processing stool cultures: another step towards automation in the microbiology laboratory. Enferm Infecc Microbiol Clin 2005; 23:58–61.

5. Sánchez-Sánchez MA, de Rafael L, Baquero F, Cantón R. Evaluation of the ROBOBACT system for automatic processing of stool cultures. Enferm Infecc Microbiol Clin 2003;21:463.

6. Daza R, Gutiérrez J, Piédrola G. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. Int J Antimicrob Agents 2001;18:211–215.