JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2000, p. 2870–2872 Vol. 38, No. 8
0095-1137/00/$04.00+0


# Detection of Significant Bacteriuria by Automated Urinalysis Using Flow Cytometry

HIROSHI OKADA,[1]* YUTAKA SAKAI,[1] SHIGENORI MIYAZAKI,[1] SOICHI ARAKAWA,[1] YUKIO HAMAGUCHI,[2] AND SADAO KAMIDONO[1]

*Department of Urology, Kobe University School of Medicine,[1] and The Sysmex Corp.,[2] Kobe, Japan*

Received 3 January 2000/Returned for modification 18 March 2000/Accepted 29 May 2000

**A new flow cytometry-based automated urine analyzer, the UF-50, was evaluated for its ability to screen urine samples for significant bacteriuria. One hundred eighty-six urine specimens from patients attending an outpatient clinic of a university-based hospital were examined. The results obtained with the UF-50 were compared with those obtained by conventional quantitative urine culture. The UF-50 detected significant bacteriuria with a sensitivity of 83.1%, a specificity of 76.4%, a positive predictive value of 62.0%, a negative predictive value of 90.7%, and an accuracy of 78.5%. These results are comparable to those obtained by previously reported screening procedures. Besides detecting significant bacteriuria, the UF-50 can also perform routine urinalysis, including measurement of concentrations of red blood cells, white blood cells, epithelial cells, and casts, within 70 s. This capability renders this new flow cytometry-based urine analyzer superior to previously reported rapid screening methods.**

Urinary tract infection is diagnosed on the basis of symptoms, signs, and urinalysis. For a final diagnosis, quantitative measurement of bacterial concentrations in the urine is mandatory. However, quantitative bacterial culture is very time-consuming and expensive. Therefore, clinicians have sought a reliable screening method for differentiation of urine samples which contain significant numbers of bacteria from those which do not. Approximately 80% of urine cultures are negative (5). If positivity of urine cultures can be predicted before urine culture is performed, the time and cost expended on examination of negative cultures can be avoided.

We have been working with an industrial company to create a new device that can perform urinalysis automatically. Advancements in computer technology make it possible to use flow cytometric techniques to perform automated urinalyses. A new automatic urine analyzer, the UF-50 (Sysmex Corporation, Kobe, Japan), is the first compact machine that quantitatively measures the concentrations of red blood cells (RBCs), white blood cells (WBCs), epithelial cells (ECs), casts, and bacteria in urine samples with high reproducibility (H. Okada, Y. Nakano, Y. Sakai, S. Miyazaki, M. Fujisawa, S. Arakawa, Y. Hamaguchi, and S. Kamidono, submitted for publication).

In the present study, we investigated the feasibility of using the UF-50 to predict the outcome of quantitative urine cultures.

## MATERIALS AND METHODS

**Urine specimens.** Urine specimens were collected from patients who attended the outpatient clinic of Kobe University Hospital. Patients with urinary diversions or those with orthotopic neobladder with bowel segments were excluded, and all patients had normal urinary tract anatomies.

A sterile container with a wide opening was used to collect midstream urine. Female patients were asked to wipe their external genitalia with a wet tissue before urinating. Ten milliliters of each specimen was used for routine urinalysis including qualitative measurement of protein and sugar and microscopic examination of centrifuged urinary sediments. The rest of the specimen was processed for automated urinalysis with the UF-50 and by urine culture immediately following collection.

* Corresponding author. Mailing address: Department of Urology, Kobe University School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Phone: 81 78 382 6155. Fax: 81 78 382 6169. E-mail: okada@med.kobe-u.ac.jp.

**Semiquantitative urine culture.** Semiquantitative urine cultures were performed by procedures based on the recommendation of Cintron (4) with cystine-lactose-electrolyte-deficient (CLED) agar (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan). Most urinary pathogens can grow on CLED agar, and it can inhibit the swarming of *Proteus* species. A 0.001-ml loop was used for inoculation of urine samples. Bacterial concentrations were determined by a single technician and were expressed as the numbers of CFU per milliliter.

Significant bacteriuria was assessed by the following criteria. Samples were considered positive if they contained $\geq 10^5$ or $10^4$ to $<10^5$ CFU of urinary pathogens/ml of pure culture. Samples were also considered positive when two or more potentially pathogenic bacterial species with individual counts of $>10^4$ CFU/ml were isolated or when the count for one organism was $>10^4$ CFU/ml and it was clearly predominant (i.e., present in numbers at least 10-fold greater than the numbers of the other organisms present). The microorganisms isolated were identified by standard biochemical procedures (5).

**Automated urinalysis and detection of significant bacteriuria with the UF-50.** Automated urinalysis was performed with the UF-50. In brief, 2 ml of urine was mixed well, and then 400 μl of the sample was diluted with 1,160 μl of diluent and 40 μl of a mixture of two distinct fluorescent staining dyes (carbocyanine and phenanthridine). This mixture was hydrodynamically focused and was passed through the sheath flow cell, and then each sample was illuminated with an argon laser beam.

Individual cells and formed elements in the urine fluoresced to various degrees. The scattered light intensity and fluorescent intensity of each cell and formed element in the urine were converted into electrical signals. Thus, four parameters were measured simultaneously in each sample: forward-scattered light intensity, forward-scattered light intensity pulse width, fluorescent light intensity, and fluorescent pulse width. After data reduction, the numbers of RBCs, WBCs, ECs, casts, and bacteria in 1 μl were recorded on a screen, and a hard copy of the results was obtained. Problematic specimens which contained pathological casts, yeast-like organisms, or sperm were identified and recommended for manual microscopic examination. The sample was assessed as containing a significant number of bacteria if the WBC concentration exceeded 5 cells/ml and the bacterial forward-scattered light intensity exceeded 12 arbitrary units (based on a referential unit of light intensity in the UF-50).

**Statistical analysis.** Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by the method developed by Ransohoff and Feinstein (7). The false-positive rate, false-negative rate, and accuracy were also calculated. Each calculated value was expressed as a percentage.

## RESULTS

One hundred eighty-six urine samples were evaluated with the UF-50 and by semiquantitative urine culture. Of these, 100 were from male patients and 86 were from female patients. The incidence of positive cultures was 31.7% (59 of 186). The microorganisms isolated follow, with the number of isolates indicated in parentheses: *Escherichia coli* ($n = 21$), *Pseudomonas aeruginosa* ($n = 12$), *Enterococcus* spp. ($n = 7$), coagulase-

TABLE 1. Correlation between significant bacteriuria detection with the UF-50 and by semiquantitative urine culture[a]

| Result obtained with UF-50 | No. of urine samples with the following culture result[b]: | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 49 (*a*) | 30 (*b*) | 79 |
| Negative | 10 (*c*) | 97 (*d*) | 107 |
| Total | 59 | 127 | 186 |

[a] The criteria for significant bacteriuria are described in the text.

[b] Sensitivity was calculated as $[a/(a + c)] = 49/59$ [83.1%], specificity was calculated as $[d/(b + d)] = 97/127$ (76.4%), PPV was calculated as $[a/(a + b)] = 49/79$ (62.0%), NPV was calculated as $[d/(c + d)] = 97/107$ (90.7%), false-positive rate was calculated as $[b/(a + b + c + d)] = 30/186$ (16.1%), false-negative rate was calculated as $[c/(a + b + c + d)] = 10/186$ (5.4%), and accuracy was calculated as $[(a + d)/(a + b + c + d)] = 146/186$ (78.5%).

negative *Staphylococcus* ($n = 5$), *Staphylococcus aureus* ($n = 4$), *Klebsiella pneumoniae* ($n = 3$), *Candida albicans* ($n = 3$), *Citrobacter freundii* ($n = 3$), *Stenotrophomonas maltophilia* ($n = 2$), *Morganella morganii* ($n = 2$), *Serratia marcescens* ($n = 2$), *Proteus vulgaris* ($n = 1$), and a *Corynebacterium* sp. ($n = 1$). Of the 59 positive urine samples, a single pathogen was isolated from 35 samples, two pathogens were isolated from 15 samples, and three pathogens were isolated from 1 sample. The correlation of the results obtained with the UF-50 and by semiquantitative urine culture was evaluated in terms of sensitivity (83.1%), specificity (76.4%), PPV (62.0%), and NPV (90.7%) (Table 1).

## DISCUSSION

The purpose of rapid screening of urine is to eliminate negative specimens quickly and cheaply, allowing the laboratory to direct more effort into positive specimens. For physicians, this should mean prompt reporting of normal specimens and improvement in the quality of patient care. Over the past decade several rapid screening methods have been reported, including semiautomated microscopy combined with reagent strip chemical determinations (Yellow IRIS) (1), reagent strip chemical determinations alone (Clinitek 200) (2), detection of catalase activity (Uriscreen) (6), and microscopic examination of Gram-stained unspun urine (3). Results for the detection of significant bacteriuria by these methods were compared with reference urine culture results. The Yellow IRIS showed a sensitivity of 92.8%, a specificity of 60.1%, a PPV of 47.9%, and an NPV of 95.5%. The Clinitek 200 was reported to have a sensitivity of 96.9%, a specificity of 75.7%, a PPV of 52.7%, and an NPV of 98.9%. The Uriscreen showed a sensitivity of 65.2%, a specificity of 85.7%, a PPV of 57.7%, and an NPV of 89.2%. Microscopic examination of Gram-stained unspun urine showed a sensitivity of 96.0%, a specificity of 99.2%, a PPV of 97.6%, and an NPV of 98.7%. Results vary widely between studies. Several factors probably contribute to this divergence, including different bacterial detection methods, different definitions of significant bacteriuria ($\geq 1 \times 10^3$ CFU/ml, $\geq 1 \times 10^5$ CFU/ml, or $5 \times 10^4$ CFU/ml, the last of which was the same as ours), and different patient populations (392 inpatients and outpatients, 1,020 outpatients, 200 children undergoing urodynamic evaluation, 500 inpatient and outpatients in the four studies, respectively).

While the methods mentioned above can be used to perform either bacterial detection alone (1, 6) or bacterial detection and qualitative analysis for the presence of RBCs and WBCs, the UF-50 is able to perform quantitative urinalysis. The UF-50 can assess RBC and WBC concentrations and can also measure the concentrations of ECs and casts. This capability renders tests with the UF-50 superior to tests by previously reported rapid screening methods. For the screening method, the time needed to perform the analysis is the other important factor. The UF-50 can perform quantitative urinalysis, including detection of significant bacteriuria, and provide a full printed report within 70 s. Physicians can obtain information on the probability of a positive culture very quickly, and unnecessary urine cultures can be avoided. Consideration of patient symptoms in combination with urinalysis results and detection of significant bacteriuria with the UF-50 can assist in clinical decision making regarding the initiation of treatment for urinary tract infection.

Using the UF-50, one technologist can handle 200 samples in less than 5 h (data not shown). We estimate that use of the UF-50 for screening could eventually save one technologist assigned to urinalysis and urine culture at the Kobe University Hospital.

In the present study, the UF-50 flow cytometry-based fully automated urine analyzer was used to detect significant bacteriuria in an outpatient population attending a university-based hospital. The patients suffered from urothelial malignancies (52.7%), stone disease (19.7%), neurogenic bladder or benign prostatic hypertrophy (13.3%), and other diseases (14.3%). Examination of patient records revealed that the majority of patients found to have significant bacteriuria suffered from complicated infections.

Examination of 10 samples which had been reported to be false negative by the UF-50 showed that the results for these samples were borderline (WBC count, <4/ml in for 4 samples; forward-scattered light intensity, 11 arbitrary units for 6 samples). The bacterial species detected were *Escherichia coli* ($n = 4$), *Pseudomonas* spp. ($n = 2$), *Staphylococcus aureus* ($n = 2$), *Enterobacter* sp. ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *Candida albicans* ($n = 1$), and coagulase-negative *Staphylococcus* ($n = 1$).

There were 30 false-positive samples. Examination of patient records revealed that 18 of these patients with false-positive results had borderline numbers of bacteria ($1.4 \times 10^3$ to $1.8 \times 10^3$ CFU/ml). Eight patients were under treatment with antimicrobial agents, and this might hamper the growth of bacteria on CLED agar. Five patients had indwelling double-pigtail ureteral catheters and had many ECs, WBCs, and debris. The UF-50 might mistake this small debris for bacteria. Thus, the characteristics of urine samples may affect the results obtained with the UF-50.

It is generally accepted that the higher the NPV and the lower the false-negative rate, the more suitable the method is for screening. However, changing the settings to reduce the number of false-negative results would be expected to increase the number of false-positive results. To improve the false-negative and false-positive rates, we are now changing the technology to detect bacteria more accurately and are testing the feasibility of use of the new instrument with a wide variety of patient populations.

## ACKNOWLEDGMENTS

We gratefully acknowledge T. Matsumoto and M. Kamo for technical assistance.

This study was partly supported by the grant from Sysmex Corp.

## REFERENCES

1. **Barlett, R. C., D. A. Zern, I. Ratkiewicz, and J. Z. Tetreault.** 1992. Screening for urinary tract infection with the Yellow IRIS. Lab. Med. **23:**599–602.
2. **Bowman, R. A., and T. V. Riley.** 1991. Evaluation of Clinitek 200 and Rapi-

matII/T for screening for urinary tract infection. J. Clin. Pathol. **44:**58–60.
3. **Cardoso, C. L., C. B. Muraro, V. L. D. Siqueira, and M. Guilhermetti.** 1998. Simplified technique for detection of significant bacteriuria by microscopic examination of urine. J. Clin. Microbiol. **36:**820–823.
4. **Cintron, F.** 1992. Initial processing, inoculation, and incubation of aerobic bacteriology specimens, p. 1.4.10–1.4.11. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Kellogg, J. A., J. P. Manzella, S. N. Shaffer, and B. B. Schwartz.** 1987. Clinical relevance of culture versus screens for the detection of microbial pathogens in urine specimens. Am. J. Med. **83:**739–745.
6. **Palmer, L. S., I. Richards, and W. Kaplan.** 1997. Clinical evaluation of a rapid diagnostic screen (Uriscreen) for bacteriuria in children. J. Urol. **157:**654–657.
7. **Ransohoff, D. F., and A. R. Feinstein.** 1978. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. N. Engl. J. Med. **299:**926–930.