Evaluation of the Sysmex UF-1000i for the Diagnosis of Urinary Tract Infection

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Key Words: Sysmex UF-1000i; Flow cytometry; Urinary sediment examination; Urinary tract infection

Abstract

Diagnosis of urinary tract infection (UTI) is primarily done by microbiologic culture, which is time-consuming and can produce false-positives and false-negatives. Flow cytometry allows for rapid screening of many samples and eliminates culturing. We analyzed the Sysmex UF-1000i (TOA Medical Electronics, Kobe, Japan) for accuracy in identifying RBCs and WBCs, casts, bacteria, and epithelia. We also evaluated its precision, linear estimation of results, carryover contamination rate, and anti-interference. UF-1000i agreement with manual counting was approximately 95% for RBCs and WBCs, epithelia, and casts. Its coefficient of variation for bacteria ranged from 4.7% to 15.2%. UF-1000i screening for UTIs exhibited great sensitivity (97%), specificity (79%), positive predictive value (70%), negative predictive value (99%), and accuracy (85%). The negative predictive value remained high even with complex UTI samples. The Sysmex UF-1000i shows great promise in excluding more than 50% of true-negative samples, improving detection efficiency, and reducing laboratory costs.

Urinary tract infection (UTI) has been cited as the most common bacterial infection, and it may have serious complications for children, people with diabetes, elderly people, and people with immune compromise.1,2 It is diagnosed primarily by urine culture and clinical manifestations, with microbiologic culture still the diagnostic “gold standard,”3 despite being time-consuming, labor-intensive, and prone to false-negative or false-positive results.4-6 Flow cytometry has long been recognized as capable of identifying bacteria,7 and the Sysmex UF-100 automated urine particle flow cytometer (TOA Medical Electronics, Kobe, Japan), in use since 1997, has shown increasing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy in screening for UTI.4,8-11

Recently, the UF-100 was updated with an improved model, Sysmex UF-1000i. The UF-1000i uses a semiconductor laser (λ 635 nm) with forward and side scatter detection and a single DNA dye for its 2 counting channels, 1 for sediment analysis and 1 for microbes. When the fluorescein-stained urine sample is inserted into the UF-1000i sheath-flow cross-flow pool, it is irradiated by the laser beam, and particles in the urine sample produce signals in the forward scattering light, side scattering light, and side fluorescence signal. Those signals are converted to optoelectronic signals so components can be identified, counted, classified, and analyzed. Bacteria are counted via a special bacterial examination channel, and interference with RBCs is prevented.

We hypothesized that, compared with its predecessor, the UF-1000i would have better sensitivity, specificity, PPV, NPV, and accuracy in identifying the 5 formed components of urine (RBCs, WBCs, casts, bacteria, and epithelial cells) and...
evaluated its precision, linear estimation of results, carryover contamination rate, and anti-interference.

**Materials and Methods**

**Specimens**

Random urine samples (n = 313) were obtained from inpatients and outpatients of our hospital from August 8, 2007, to March 17, 2008. During the same period, the microbiology room of the hospital Department of Laboratory Medicine obtained 368 urine samples from various departments for urine culture, using disposable urine collection cups with covers. Sample collection was performed in strict accordance with the operational standards of clean midstream urine collection. The study was approved by the hospital institutional review board and was in accordance with the Helsinki Declaration of 1975. No informed consent was required.

**Urinalysis for UTI Diagnosis**

All samples were inoculated within 1 hour after collection. Clean midstream urine was fully mixed and was inoculated onto the blood plate and MacConkey flat plate using a 0.001-mL quantitative inoculation ring. Samples were cultured in an incubator at 37°C for 24 hours, at which time colony counting and Gram staining were carried out. Gram-negative bacteria of more than 10⁵ colony-forming units (CFU)/mL and gram-positive bacteria of more than 10⁴ CFU/mL indicated a UTI, as per domestic and National Committee for Clinical Laboratory Science standards for diagnosis. This is more rigorous than the international standard of more than 10⁵ CFU/mL for gram-negative and gram-positive bacteria.

**Manual Examination of Urine Samples**

Two senior technicians used the FAST-READ 10 counting board (Immune Systems, London, England) to count RBCs, WBCs, casts, bacteria, and epithelial cells manually in accordance with the urine sample examination standard. The RBC and WBC counts were divided into 7 levels: 0 to 50, 51 to 100, 101 to 200, 201 to 300, 301 to 500, 501 to 800, and 801 to 1,000/μL. The RBC count was divided into 4 levels: 0 to 2, 2.1 to 5, 5.1 to 10, and 10.1 to 15. The WBC count was divided into 4 levels: 0 to 15, 15.1 to 25, 25.1 to 40, and 40.1 to 60/μL. The cast count was divided into 4 levels: 0 to 3, 3.1 to 6, 6.1 to 9, and 9.1 to 12/μL. Technicians were blinded to the UF-1000i results.

**Examination of Urine RBCs, WBCs, Casts, Bacteria, and Epithelial Cells Using the UF-1000i**

For analysis, a 10-mL urine sample was collected and put into a test tube. The test tube was put into the matching test-tube rack of the instrument, and the instrument examined the samples automatically. Cells were differentiated using multiple indexes, including the forward scattering light signal, the side scattering light signal, and fluorescence signal, and cells of similar size, such as crystals and RBCs and also small round cells and WBCs were identified and classified. The pathologic casts and casts without inclusions are differentiated according to the level of fluorescence distribution width. The machine provides an automatic count of RBCs, WBCs, casts, bacteria, and epithelial cells.

**UF-1000i for UTI Diagnosis**

To determine the amount of gram-negative bacteria and gram-positive bacteria, we converted the domestic standard for a diagnosis of UTI to one appropriate for the UF-1000i. To do this, we first set the colony count of the quantitative bacterial culture of the regular clean midstream urine sample (with urine that has stayed in the bladder for 4-6 hours) at 10⁵/mL or more, set the bacterial count at 10⁵/mL or more, and set the yeast-like fungi count at 100/μL or more. Then we set the number of WBCs in the sediment of the centrifuged clean midstream urine at more than 10/HP for samples from patients with UTI symptoms. Because the UF-1000i analyzer can convert between HP and microliters (1:5.6), the number of WBCs was set to more than 56/μL.

**Examination of UF-1000i Precision**

The RBCs, WBCs, casts, bacteria, and epithelial cells in the 313 random fresh urine samples were examined manually, then examined using UF-1000i for the aforementioned 5 components. Each sample was continuously examined 11 times. The results of the last 10 examinations were recorded and analyzed.

**Linear Estimation of the UF-1000i Examination Results**

A high-value sample with values close to the expected upper limit (RBCs, 10,967/μL; WBCs, 5,100/μL) was selected and diluted at ratios of 1:4, 1:16, 1:64, 1:256, and 1:1,024 in negative urine (all indexes near 0). The measured values were compared with the theoretical values, and the correlation coefficient was used to estimate linear correlation.

**Examination of the Carryover Contamination Rate of the UF-1000i**

One fresh urine sample with high values and one fresh urine sample with low values were randomly selected and the aforementioned 5 indexes determined by UF-1000i. In the high-value urine sample, components were as follows: RBCs, 10,807/μL; WBCs, 3,197/μL; casts, 23/μL; bacteria, 91,301/μL; and epithelial cells, 516/μL. The low-value fresh urine sample contained the following: RBCs, 27.7/μL; WBCs, 38.0/μL; casts, 1/μL; bacteria, 92/μL; and epithelial cells, 2/μL. To assay carryover contamination, first, the high-value sample
was analyzed 3 times continuously using the UF-1000i. Then, the low-value sample was examined 3 times without modifying any instrument settings or changing the pipette. The carryover contamination rate was evaluated by calculating the effect of the high-value sample examination on the results of the low-value sample, using the following formula:

$$\frac{w - x}{y - z} \times 100\%$$

where w equals the first-time result of the low-value sample, x equals the third-time result of the low-value sample, y equals the third-time result of the high-value sample, and z equals the first-time result of the high-value sample.

Evaluation of the Anti-interference Performance of the UF-1000i

Venous RBCs were added into a normal fresh urine sample to prepare the hematuria sample with maximum expected concentrations of RBCs. The actual concentration of RBCs was counted manually using a counting board as described. This hematuria sample was diluted in normal urine using the doubling dilution method (dilution rate of 2). Interfering factors (bacteria and yeast-like fungi) were added to the samples at different dilution rates, and the UF-1000i was used to measure the RBC count. The actual measured RBC count was compared with expected values to evaluate the antijamming performance of the UF-1000i.

Statistical Analysis

The Cohen $\kappa$ was used on dichotomous data to establish that agreement exceeded that expected under the null hypothesis of random ratings. Yerushalmy models were constructed to indicate presence of bacteria as causative for UTI.16 The correlation coefficient was used to estimate the linear correlation of theoretical vs actual counts of RBCs and WBCs as measured by the UF-1000i. Data were analyzed using STATA version 9.0 (StataCorp, College Station, TX), and a $P$ value less than .05 indicated statistical significance.

Results

UF-1000i vs Manual Counting

All clinical urine samples ($n = 313$) examined by UF-1000i and manual counting were considered consistent if the difference between the results of the 2 examination methods was within 1 SD. The RBC consistency rate was 95.11%, the WBC consistency rate was 96.70%, and the epithelial cell consistency rate was 94.15%. Except for casts, all $\kappa$ values were more than 0.81, indicating good correlation between the 2 methods. The consistency rate for casts was 95.79% with $\kappa$ value of 0.26, suggesting little correlation between the 2 methods Table 1.

UF-1000i for Identifying Gram-Positive and Gram-Negative Bacteria

Among the 368 urine culture specimens, 127 showed positive results, for a positive rate of 34.5%. Among the 132 bacterial strains isolated and identified by microbial culture, 66 were gram-negative bacteria (49.3%), 36 were gram-positive bacteria (26.9%), and 30 were fungi (22.4%). The most common microorganisms identified by the UF-1000i from a urine sample (in order of frequency) were as follows: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Proteus mirabilis (gram-negative bacteria); Enterococcus faecium, Enterococcus faecalis, Staphylococcus haemolyticus, Staphylococcus epidermidis, and Staphylococcus aureus subsp aureus (gram-positive bacteria); and Candida albicans and Candida tropicalis (yeast). UF-1000i UTI detection was compared with the results of microbial culture. The sensitivity, specificity, PPV, NPV, and accuracy of UF-1000i for UTI diagnosis were calculated (WBC >56/μL or bacteria >105/mL or yeast-like fungi >100/μL).7 The sensitivity was 0.86, specificity was 0.95, PPV was 0.91, NPV was 0.94, and the consistency rate was 0.91.

UF-1000i Precision

Precision of the UF-1000i in identifying the 5 formed components in urine is provided in Table 2. The coefficient of variation (CV) for detecting RBCs was 3.5% to 5.3% (25.16-206.80/μL); for detecting WBCs, it was 1.7% to 8.9% (20.93-488.30/μL); for bacteria, it was 4.7% to 15.2% (110.40-78,781.00/μL); for casts, it was 8.4% to 16.0% (5.64-21.31/μL); and for epithelial cells, it was 4.8% to 19.7% (13.88-119.33/μL).

Linear Assessment of UF-1000i Examination

The correlation coefficient was used to estimate linear correlation of UF-1000i Figure 1. The linear estimate of the UF-1000i for RBCs was $r = 1.000$ ($P < .05$), and for WBCs, it was $r = 1.000$ ($P < .01$).
The Carryover Contamination Rate of the UF-1000i

The carryover contamination rates of RBCs, WBCs, epithelial cells, casts, and bacteria were 0.07%, –0.03%, –0.02%, 0.49%, and –0.04%, respectively.

Anti-interference Performance of UF-1000i Examination

Results of the hematuria sample examined by UF-1000i with bacterial interference are shown in Table 3. Correlation coefficient analysis showed no difference among theoretical values in RBC at interference levels of 10⁴ to 10⁷/mL (P < .0001). Results of the hematuria sample examined by the UF-1000i with fungal interference are shown in Table 4. Correlation coefficient analysis showed that concentrations of interfering factors of 100 to 400/μL did not cause statistically significant interference with RBC examination (P < .0001).

Discussion

Urinary sediment examination is an important component of urine analysis, greatly assisting with clinical diagnosis, therapeutic monitoring, and health screenings. In our analysis, the UF-1000i achieved a CV for RBCs of at least 5.3%. This correlates well with the recent analysis by Shayanfar et al., who found rates of 6.5% and 5.5% for the Iris iQ200 (Iris Diagnostics, Chatsworth, CA) and the UF-100, respectively. We similarly found the newer flow cytometer to outperform the earlier device. Ozdem et al. found CVs of 6.35% to 12.18% for WBCs using the UF-100 at different concentrations; our range was 1.7% to 8.9%.

In our study, sensitivity was 86.0%, specificity was 95.0%, PPV was 91.0%, NPV was 94.0%, and consistency was 91%. False-negatives can result from other systemic infections, antibiotic use close to the time of the test, and delay in processing. For the dipstick test, false-negatives can result from abnormal pH levels, proteinuria, the presence of certain metabolites or vitamin C, and even test strip error. The cultures of the 2 false-negative samples were S aureus and E faecium. The UF-1000i bacterial counts were 10⁴ to 10⁵/mL. Although those numbers met the clinical criteria of positive infection with cocci, they did not reach the positive value set in this study and were considered negative. Among the 52 false-positive samples, the WBC count exceeded 56/μL in 22 samples, perhaps because microbial culture of the urine
failed, the patient had been taking antibiotics before sampling, or there was a lack of bacteriuria, sometimes reported in UTI. In addition, the instrument gave a “review” signal for 10 samples, suggesting a certain degree of interference with the analysis.

In a study of the UF-100 to analyze peritoneal dialysis fluid, Penders et al. combined WBC count, bacterial channel count, and total protein measurement to find a sensitivity of 75.0% (range, 47.6%-92.6%) and a specificity of 72.2% (range, 46.5%-90.2%) for diagnosing peritonitis. As others have suggested, the value of the UF-1000i lies not in replacing laboratory analysis, but in reducing the number of samples that require laborious manual counting. The UF-1000i shows great diagnostic potential for UTI screening, diagnosing other renal and urologic conditions, and examination of even cerebrospinal and other fluids.

Urinary sediment analysis requires a variety of equipment and methods, and interfering factors can produce great analytic error and differences between laboratories and slow automation progress. Three national urine analysis seminars developed by the Clinical and Laboratory Standards Institute, European Confederation of Laboratory Medicine, and Japanese Research Institute on Urinary Tract Infection, results of urinary sediment examination should be reported quantitatively. The UF-1000i automated urine particle flow cytometer can meet these requirements and classify a large number of urine particles quickly. In this sample, UF-1000i identified 65.5% of 386 samples as negative, in keeping with standards seen in the literature for the UF-100. The consistency rate for casts was 95.79% with a $\kappa$ of 0.26, suggesting little correlation between the two methods. This result may have been influenced by the high proportion of samples with low cast values and the low proportion with high cast values, which hampered statistical understanding (Table 2).

The sensitivity of the UF-1000i for UTI screening was 97%, the specificity was 79%, the PPV was 70%, the NPV was 99%, and the accuracy was 85%. The NPV was high when the results of WBC, bacteria, and yeast-like fungi were combined for UTI screening, which may help the clinical laboratory filter out more than 50% of true-negative samples, improving detection efficiency and reducing laboratory costs.

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Acknowledgment: We are grateful to Longquan Gu, MD, Ruijin Hospital, Shanghai Jiao Tong University, and Daming Jin, MD, Shanghai Clinical Laboratory Center for assistance in the evaluation of the UF-1000i; and we thank the microbiology room staff of our Department of Laboratory Medicine for their support.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Bacterial Interference With the RBC Count*</th>
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<tr>
<td>Expected Concentration of RBCs (μL)</td>
<td>Concentration of Interfering Bacteria</td>
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<td></td>
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* Results are given as means.

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<th>Table 4</th>
<th>Interference of Yeast-like Fungi With the RBC Count*</th>
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<tr>
<td>Expected Concentration of RBCs (μL)</td>
<td>Concentration of Yeast-like Fungi (μL)</td>
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<tr>
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</table>

* Results are given as means.

References

Wang et al: UF-100i for UTI Diagnosis


