Automated Urinalysis

Evaluation of the Sysmex UF-50

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Abstract

We assessed the Sysmex UF-50 for reproducibility of results and carryover rate by performing between- and within-run precision analyses on 315 urine samples, evaluated the feasibility of using the UF-50 to measure urinary cellular and noncellular components by comparing results from the UF-50 with results of manual urinalysis using the Kova system, and performed side-by-side comparison of the within-run reproducibility from the UF-50, the UF-100, and the Kova system. Results from the UF-50 and UF-100 were highly reproducible, and the carryover rate was 0.5% or less for the urinary components. In between-run precision assays, the coefficients of variation for UF-50 results for all cellular components were less than 10%. The agreement (gamma statistics) between values from the UF-50 and the Kova system was excellent for RBC, WBC, and bacterial counts. The cell counts from the UF-50 for RBCs, WBCs, epithelial cells, and bacteria were 52%, 63%, 54%, and 110%, respectively, of those measured by manual urinalysis. The UF-50 performed quantitative analysis in 72 seconds, compared with 330 seconds for manual methods. The UF-50 is suitable for the first screening to detect hematuria, pyuria, and bacteriuria.

Urinalysis is a fundamental laboratory examination that provides valuable diagnostic information in a variety of diseases. It usually is performed by microscopic examination of centrifuged urinary sediment by trained personnel. Although manual analysis procedures are standardized, several factors can affect the accuracy and reproducibility of the analytic results.1 Attempts have been made to reduce the variation of manual analysis involving the use of uncentrifuged samples and automation of urinalysis. The Kova system (ICL Scientific, Fountain Valley, CA) is a manual technique that uses unspun urine samples and is the procedure recommended by the Urinary Tract Infection Study Group in Japan for quantitative urinalysis.2 Reflectance photometry and image analysis have been used for automation, but neither has provided results comparable to manual analysis.3-5

CBC counts also were performed by manual analysis using a hemocytometer, but automated analyzers that use flow cytometry have been developed to take over this task.6 The same technology has been applied to count components of urine (eg, RBCs, WBCs, epithelial cells [ECs], crystals, casts, and bacteria). The first flow cytometry–based urinalysis device, the UF-100, was produced by Sysmex (Kobe, Japan).7,8 The UF-50 was developed as a more compact version of the UF-100 (Sysmex). As part of the development of the UF-50, changes were made to the image analysis algorithm to improve morphometric reproducibility. In the present study, we evaluated the reproducibility of results from the UF-50, compared with those from manual microscopic urinalysis with chamber slides using unspun urine samples from a urologic outpatient clinic at a university-based hospital to test the feasibility of using the device.
Materials and Methods

Urine Specimens

Urine specimens were provided by patients who attended the urologic outpatient clinic between September and December 1998 at the Kobe University Hospital, Kobe, Japan, as part of a routine examination. Female patients were asked to wipe their genitalia with wet tissue and used a sterile container with a wide opening to collect midstream urine. A portion of each specimen (10 mL) was poured into a 15-mL conical tube with a screw cap (Becton Dickinson, Lincoln Park, NJ) and centrifuged for 5 minutes at 1,500 rpm with a centrifuge (KN-70, Kubota, Tokyo, Japan) at room temperature. The sediment that formed was used in routine urinalysis by the urology outpatient clinic. The remainder of the specimen was used in the study. Automated and manual microscopic urinalyses were performed within 1 hour of specimen collection. To reduce interobserver variation, one technician performed all manual urinalyses, while another performed all automated urinalyses. The use of such discarded material was reviewed and approved by the institutional review board committee at the Kobe University School of Medicine, Kobe, Japan.

Urinalysis With the Kova System

The Kova system is the standard method for manual analysis of urine samples at our institution. In brief, the urine was mixed thoroughly with a Pasteur pipette, and a drop was placed into the chamber of a Kova Glassic Slide 10 with Grids (ICL Scientific, Fountain Valley, CA) by capillary action and examined by microscope (BH-2, Olympus, Tokyo, Japan). Each chamber on the slide was divided into 81 smaller chambers, each containing 0.011 mL of urine. The number of RBCs, WBCs, ECs, casts, and bacteria within 4 or 5 small chambers was counted at ×400 magnification, and the average number of cells and casts per small chamber was multiplied by 90 to give the number per microliter.

Automated Urinalysis

The procedures for analysis were identical to those for the UF-100. However, the analytic data were presented only as numeric data for the UF-50, while scattergrams also were presented for the UF-100. In brief, 2 mL of urine was mixed well, and 400 µL of the sample was diluted with 1,160 µL of diluent and 40 µL of a mixture of 2 fluorescent staining dyes (0.14% carbocyanine, 0.04% phenanthridine, and 99.82% ethylene glycol). The mixture was then focused hydrodynamically, passed through the sheath flow cell, and illuminated by an argon laser beam. With this method, individual cells and casts in the urine fluoresce to varying degrees. The scattered light intensity and fluorescent intensity of each cell and cast were converted into electrical signals by a photomultiplier. Thus, 4 variables 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 number of RBCs, WBCs, ECs, casts, and bacteria in 1 µL was recorded on the screen, and a hard copy of the results was obtainable.

Between-Run Precision

The manufacturer provided quality-control samples containing 5-µm, 8-µm, 25- to 40-µm, 38- to 75-µm, and 3-µm diameter particles to mimic the size of RBCs, WBCs, ECs, casts, and bacteria, respectively. These samples were measured on 15 separate days throughout the course of the study, and the coefficients of variation (CVs) were calculated for each component.

Within-Run Precision

To test the consistency of results, repeated measurements were made on samples using the UF-50, the UF-100, and the Kova system. Measurements with the UF-50 and the UF-100 were repeated 18 to 20 times, and measurements using the Kova system were repeated 10 times. The precision of each measurement method was assessed by the CV obtained.

Linearity Assessments

Samples containing decreasing amounts of cellular and noncellular components were prepared by diluting representative urine samples with control urine in a stepwise dilution (1:1, 1:2, 1:4, 1:8, 1:16, 1:32); control urine was a mixture of urine from 2 healthy volunteers that had been filtered through a 0.22-µm filter (Becton Dickinson) to remove cellular and noncellular particles. Linearity was determined by analyzing in triplicate specimens and their dilutions, and the slope and intercept to expected value were determined.

Analysis of Carryover

Four urine specimens, which contained various amounts of cellular and noncellular components, were used to assess between-specimen carryover. Each specimen was analyzed in triplicate, followed by 3 blank specimens of control urine. The carryover rate for cellular and noncellular components was calculated by the following formula:

\[
\text{Carryover Rate (\%)} = \left(\frac{(B1 - B3)}{(S3 - B3)}\right) \times 100
\]

where B1 is the first measured value of blank; B3, the third measured value of blank; and S3, the third measured value of the specimen.

Measurement of Time Needed for Urinalysis

The time needed to perform automated urinalysis was defined as the time between the aspiration of urine samples...
and the presentation of the results on the screen. The time needed to perform manual urinalysis was defined as the time between the loading of urine samples on the Kova slide and completion of differential counts of cellular and noncellular components in the urine.

Statistical Analysis

The Spearman rank correlation coefficient was used to determine the correlation between values obtained by the 2 methods; P values of less than .05 were considered statistically significant. The results from the UF-50 and the Kova system were compared by gamma statistics using SPSS for Windows (SPSS, Chicago, IL).9 Gamma is a measure of association between 2 variables measured on an ordinal level and can be thought of as the probability that a random pair of observations is concordant minus the probability that the pair is discordant, assuming the absence of numerically identical results. Gamma is symmetric and ranges between 0 and 1.

Results

A total of 500 urine specimens were collected and examined by normal urinalysis; those showing macroscopic hematuria and chyluria were excluded from the study. Urine collected in nonsterile containers and specimens smaller than 20 mL also were excluded. After exclusions, 315 urine samples were used in the study.

Linearity of Results

Dilutions of urine samples were prepared as described in “Materials and Methods.” For casts, dilutions of 1:2 and 1:4 were made from samples with 20 casts per milliliter, and specimens were analyzed in triplicate. For 4 samples with RBC concentrations of 17 to 1,459 cells per milliliter, the measured values were 99.9% to 101.3% of expected. For 4 samples with WBC concentrations of 100 to 1,872 cells per milliliter, values were 99.0% to 100.4% of expected. For 4 specimens with EC concentrations of 6 to 128 cells per milliliter, the manual values were 97.8% to 102.2% of expected. For 4 samples with bacteria concentrations of 3,464 to 93,246 cells per milliliter, results were 96.0% to 100.0% of expected, and for casts (1 sample), 100.6%.

Carryover

Analyses of carryover were performed with the UF-50. The carryover rate was determined using 4 urine specimens of various RBC, WBC, EC, cast, and bacteria counts: RBCs, 7 to 1,358/µL; WBCs, 33 to 368/µL; ECs, 15 to 130/µL; casts, 0.1 to 10/µL; and bacteria, 3,210 to 24,451/µL. Carryover ranges were 0.0% to 0.352% for RBC counts, 0.0% to 0.096% for WBC counts, 0.0% to 0.459% for EC counts, 0% for cast counts, and 0.0% to 0.201% for bacterial counts.

Between- and Within-Run Precision

The between-run precision of the UF-50 was analyzed using quality control specimens on 15 separate days. The CVs were 3.1%, 2.0%, 4.4%, 7.8%, and 9.3% for the measurement of RBCs, WBCs, ECs, casts, and bacteria, respectively.

Within-run reproducibility for the detection of RBCs, WBCs, ECs, casts, and bacteria using the UF-50, the UF-100, and the Kova system is shown in Table 1. Overall, manual urinalysis showed a higher within-run CV than automated urinalysis by the UF-50 or the UF-100, although the 3 systems tended to have greater CVs at lower concentrations.

| Table 1 |
| Within-Run Reproducibility |

<table>
<thead>
<tr>
<th>Cellular Component in Urine</th>
<th>Mean Cell Count (/µL)</th>
<th>UF-50*</th>
<th>UF-100†</th>
<th>Kova‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>12.9-15.5</td>
<td>17.3-19.8</td>
<td>82.0-96.0</td>
<td></td>
</tr>
<tr>
<td>10-100</td>
<td>6.8-6.9</td>
<td>4.4-13.6</td>
<td>26.6-570</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>1.6-4.6</td>
<td>1.2-5.1</td>
<td>12.0-34.8</td>
<td></td>
</tr>
<tr>
<td>WBCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>11.7-13.9</td>
<td>11.3-12.7</td>
<td>82.0-94.3</td>
<td></td>
</tr>
<tr>
<td>10-100</td>
<td>5.4-12.9</td>
<td>7.3-12.0</td>
<td>43.9-44.5</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>2.2-7.4</td>
<td>3.4-6.5</td>
<td>12.4-22.4</td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>12.1-74.0</td>
<td>20.0-76.8</td>
<td>0.0-210.8</td>
<td></td>
</tr>
<tr>
<td>10-25</td>
<td>9.0-14.0</td>
<td>9.7-10.1</td>
<td>72.0-95.0</td>
<td></td>
</tr>
<tr>
<td>&gt;25</td>
<td>26.5-28.6</td>
<td>11.8-18.9</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300-699</td>
<td>5.8-9.6</td>
<td>4.2-5.0</td>
<td>36.3-135.5</td>
<td></td>
</tr>
<tr>
<td>700-2,000</td>
<td>3.4-4.8</td>
<td>3.2-5.4</td>
<td>16.4-34.8</td>
<td></td>
</tr>
<tr>
<td>&gt;2,000</td>
<td>1.7-7.6</td>
<td>1.4-4.1</td>
<td>15.5-31.5</td>
<td></td>
</tr>
</tbody>
</table>

*Sysmex, Kobe, Japan.
†ICL Scientific, Fountain Valley, CA.
Comparison With Manual Results

Values measured by the UF-50 correlated well with values measured by the Kova system for RBCs, WBCs, and bacteria, and there was a significant correlation between manual and automated urinalysis for all of these components (Figure 1); the gamma statistic also approached 1. However, for ECs, the values measured by the UF-50 were poorly correlated with those measured by manual analysis (Figure 1C). Since more than 64% of specimens contained fewer than 0.3 casts per microliter, equivalent to 1 cast per low-power field, statistical analyses were not performed. The cell counts from the UF-50 for RBCs, WBCs, ECs, and bacteria were 52%, 63%, 54%, and 110%, respectively, of those measured by the Kova system.

**Figure 1** Correlation between values measured by the Kova system (ICL Scientific, Fountain Valley, CA) and the UF-50 (Sysmex, Kobe, Japan) for cellular components in urine samples. **A**, RBC counts. \( y = 0.5218x - 1.9527 \) (\( P < .01 \)); gamma = 0.959; \( n = 315 \). **B**, WBC counts. \( y = 0.6288x + 6.2461 \) (\( P < .01 \)); gamma = 0.984; \( n = 315 \). **C**, Epithelial cell counts. \( y = 0.5418x + 4.3563 \) (\( P < .01 \)); gamma = 0.596; \( n = 315 \). **D**, Bacterial counts. \( y = 1.0953x - 738.19 \) (\( P < .01 \)); gamma = 0.918; \( n = 315 \).

Statistical analysis was performed by Spearman rank correlation. The association of the values measured by the Kova and the UF-50 was assessed by gamma statistics.
Discussion

Urinalysis gives a direct indication of the state of a patient's genitourinary system and, as urine specimens are easily obtained, is performed frequently as a routine laboratory test in most clinical departments. In ordinary clinical laboratories, it is performed by microscopic observation of the sediment prepared by centrifugation of urine. However, obtaining accurate results by manual examination may be hampered by methodologic problems. To improve the precision of urinalysis, automated methods have been tried using several instruments. These instruments perform specific gravity determination and reflectance photometry of multiple dipstick test or use image analysis of the size of urine components. The Yellow IRIS (International Remote Imaging System, Chatsworth, CA) was the first device designed to visualize the cellular components in urine by combination of a video camera and a stroboscopic lamp, with image analysis used to detect and sort the particles into predetermined size ranks. However, this system still needed a technician to interpret the result of sizing.

Since the introduction of flow cytometry into hematology analyzers, CBC counts and differential cell counts have become fully automated procedures. A flow cytometer used for reticulocyte analysis (Sysmex R-1000, Toa Medical Electronics, Kobe, Japan) was tried in urinalysis to assess the feasibility of this system in developing fully automated urinalysis. A flow cytometric urinalysis analyzer, the UF-100, subsequently was reported to perform automated urinalysis successfully. The UF-50 is the latest device for automated urinalysis and uses a method similar to that of the UF-100 with improvements to the gating system and morphometric technique. The UF-50 analyzes 30,000 cellular or noncellular components in the urine per assay, while the UF-100 counts 10,000. Moreover, the algorithm was optimized to differentiate RBCs, WBCs, ECs, and bacteria correctly. The UF-100 was designed to be used in the central clinical laboratory of a hospital and requires a relatively large space. In contrast, the UF-50 is smaller and better suited to the limited space of individual outpatient clinics.

We evaluated the accuracy of urinalysis by the UF-50 by comparing the results obtained by this device with those by a manual method. Manual and automated urinalyses accurately detected a wide range of concentrations of cellular components. In addition, the carryover rate of the UF-50 was low (0.0%-0.459%), suggesting there was no substantial carryover between samples.

A between-run precision assay found the CVs for results from the UF-50 were less than 10% for all cellular components in urine. In within-run precision assays, the UF-50 showed good precision for measuring RBCs, WBCs, ECs, and bacteria in the urine; the CVs were similar to those for the UF-100 and smaller than those reported for manual analysis or found by manual urinalysis in our study. These results confirmed that the UF-50 is capable of reproducible measurement of urine cellular components in the clinically relevant range.

The former study of the UF-100 reported that it overestimated RBC and WBC counts, but the UF-50 underestimated them. In the former study, centrifuged urine samples were used; however, unspun specimens were used in the present study. The centrifugation procedure might cause substantial loss of cells in the urine. This may explain the difference observed in the present study.

Although a difference was observed for manual and automated results, it was not clinically significant. Since the analytic results obtained by both methods showed good linearity in a wide range, automated analysis can be used to indicate clinically problematic specimens. It is practical to suggest performing manual confirmation of abnormal results from the automated analysis by the UF-50.

It has been recommended that quantitative urinalysis be performed using urine samples without centrifugation. We used the Kova system without centrifugation as a standard to evaluate the feasibility of using the UF-50 for such samples. The Kova system is accepted as a useful method for detecting pyuria, and RBCs, WBCs, ECs, bacteria, and casts are reported as the number per milliliter of urine sample. This system is recommended as a standard procedure for urinalysis by the Urinary Tract Infection Study Group in Japan. Comparison of the UF-50 with manual urinalysis is sometimes difficult, but from the gamma statistics, we can conclude that the counts of cellular elements with the UF-50 are comparable to those obtained by manual urinalysis using unspun urine samples (Kova system). The results from the 2 methods were not identical; manual urinalysis tended to give larger values for RBCs and WBCs than the UF-50. The manufacturer of the Kova system reported in the user's manual that the measured results of concentrations of RBCs and WBCs were larger than those obtained by the manual method using the Fuchs-Rosenthal chamber. Both the Kova system and the Fuchs-Rosenthal chamber manual methods use counting chambers. The Kova system counts components in 0.1 µL of the urine, while manual urinalysis using the Fuchs-Rosenthal chamber counts components in 3.2 µL of urine. The results of the Kova system (y) are
expressed using the measured Fuchs-Rosenthal chamber results (x) by the following formulas:

\[ RBC, y = 1.60x + 10.76 \]
\[ WBC, y = 1.64x - 0.58 \]

This phenomenon may partly explain the reason that manual urinalysis using the Kova system tended to give larger values for RBCs and WBCs. The other possibility is that manual microscopic analysis also may detect the denatured or fragmented cellular components and result in larger values.

Some large discrepancies between the UF-50 and the Kova system in the measurement of ECs were noted. The reason for this phenomenon is not clear from the present study; however, this showed the limitation of the morphometric analysis.

The average time taken by the UF-50 to measure one sample was 72 seconds, while the Kova system took 330 seconds. From our assessment, the UF-50 performs urinalysis in a more time-saving manner than manual microscopic urinalysis, without operator interaction. Thus the UF-50 is useful as a first screening method to detect hematuria, pyuria, and bacteriuria.

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