Journal of Clinical Laboratory Analysis 22:262–270 (2008)

# Clinical Laboratory Automated Urinalysis: Comparison Among Automated Microscopy, Flow Cytometry, Two Test Strips Analyzers, and Manual Microscopic Examination of the Urine Sediments

**S. Mayo, D. Acevedo,* C. Quiñones-Torrelo, I. Canós, and M. Sancho**
*Departamento de Bioquímica, Servicio de Análisis Clínicos, Hospital Universitario Dr. Peset, Valencia, Spain*

Urinalysis is one of the habitual clinical laboratory procedures, which implies that one of the largest sample volumes currently requires significant labor to examine microscopic sediments. Different analyzers currently used to perform this task have been compared with the manual microscopic sediment examination. The Atlas Clinitek 10 (Bayer Corporation, Diagnostics Division, Tarrytown, NY) and Urisys 2400 (Hitachi Science Systems Ltd., Ibaraki, Japan) test strips analyzers and two automated urinalysis systems, Sysmex UF-100 (Sysmex Corporation Kobe, Japan) and IRIS iQ200 (International Imaging Remote Systems, Chatsworth, CA), have been considered. We assessed the concordance between the results obtained from 652 freshly collected urine samples for erythrocytes (RBC), leukocytes (WBC), squamous epithelial cells (EC), nitrites/bacteria, and crystals using the methodologies mentioned. A principal components analysis was performed in order to examine the correlation between these parameters. Instrument accuracy was also assessed. The Spearman's statistic (p) showed an adequate agreement between methods for RBC (iQ200 = 0.473; UF-100 = 0.439; Atlas = 0.525; Urisys = 0.539), WBC (iQ200 = 0.695; UF-100 = 0.761; Atlas = 0.684: Urisys = 0.620), and bacteria/nitrites (iQ200 = 0.538; UF-100 = 0.647; Atlas = 0.532; Urisys = 0.561) counts. By applying the Wilcoxon and McNemar tests, a concordance degree was found between 82–99 and 52–95% for the values obtained from the two test strips analyzers considered and from the iQ200 and UF-100 systems, respectively. From these results, we can conclude that both test strips analyzers are similar and, on the other hand, that automated urinalysis is needed to improve precision and the response time; but sometimes manual microscopic revisions are required, mainly when flags, because of crystals, are detected. J. Clin. Lab. Anal. 22:262–270, 2008.


**Key words:** automated dipstick readers; procedure agreement; routine manual diagnosis; urine analyzers

## INTRODUCTION

The aim of the routine study in clinical laboratories of urine samples is to identify and monitor renal and urinary tract illnesses. The first step of this study is based on different chemical reactions, which constitute the test strips methodology. To complete the study, the identification and count of the different cells and other particles present in urine samples are needed. This is a time-consuming process and requires a certain degree of training of the staff involved. Moreover, the precision of this study is low because of sample preparation and variations in the particle count techniques (1,2).

As previously mentioned, given the volume of urine samples that clinical laboratories receive (around 30% of the total sample volume), means that it is necessary to find a method, which speeds up their study. The introduction to the clinical practice of systems,

*Correspondence to: D. Acevedo, Departamento de Bioquímica, Servicio de Análisis Clínicos, Hospital Universitario Dr. Peset, Avenida Gaspar Aguilar, 90, 46017-Valencia, Spain.
E-mail: acevedo_del@gva.es

Received 27 May 2007; Accepted 6 March 2008
DOI 10.1002/jcla.20257
Published online in Wiley InterScience (www.interscience.wiley.com).

which carries out an automated urinalysis (automated microscopy analyzers such as the IRIS Yellow system in the mid 1980s and flow cytometry in the 1990s, which is the basis of the Sysmex-50 methodology) (3–7), was initially thought to replace the manual microscopic revisions of sediments (8). However, because of their limitations, manual sediment microscopic revision is still necessary (9). In the year 2000, guidelines for manual urine sediment study were published by the European Confederation of Laboratory Medicine (10,11).

The objective of this work was to assess the concordance degree between the two automated urinalysis systems currently available and based on such different technologies (Sysmex UF-100, Sysmex Corporation Kobe, Japan, and IRIS iQ200, International Imaging Remote Systems Chatsworth, Los Angeles, CA) and the traditional manual microscopic sediment examination to decide which analyzer would be acquired for our laboratories. At the same time, the two test strips analyzers currently used in our hospital were also compared.

## MATERIAL AND METHODS

### Samples

We studied 652 freshly collected urine samples belonging to ambulatory patients (nonhospitalized) and submitted them for diagnostic urinalysis to our laboratory. The samples were analyzed without centrifugation in the four analyzers considered, they were then centrifuged, and the routine diagnostic microscopic urinalysis was performed. All these processes were carried out within 2 hr of receiving the samples.

### Methods

#### Semiquantitative test strips analyzers

The Atlas Clinitek 10 (Bayer Corporation, Diagnostics Division, Tarrytown, NY) and Urisys 2400 (Hitachi Science Systems Ltd., Ibaraki, Japan) analyzers use a reflectance photometer as a measuring system. Ten different parameters are assessed: specific gravity (SG, measured via a built-in refractometer), pH, leukocytes (esterases), nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and erythrocytes (hemoglobin) (12,13).

The Clinitek Atlas 10 system loads 490 tests at one time for optimized reagent replacement time. It presents a sample capacity of 10 samples per rack, loading up to 200 samples per cycle. It requires a minimum volume of 2.0 mL. The throughput is one sample every 16 sec (225 samples/hr).

The Urisys 2400 system allows a fully automated operation and enhanced walk-away time with a full load of 75 samples (15 racks). It uses cassettes with 400 test strips. A minimum sample volume of 1.5–2.0 mL is required and 240 samples can be analyzed in an hour. This analyzer has a dedicated STAT position for immediate measurement of emergency samples.

#### Quantitative automated urinalysis systems

The Sysmex UF-100 performs microscopic urinalysis by flow cytometry. The cells (RBC, WBC, and epithelial cells, EC), bacteria, and casts are categorized in a multidimensional space on the basis of their size, shape, volume, and staining characteristics. Other nonquantified, but potentially pathologic, components are detected and marked as alarm, where the thresholds are defined by the user: crystals, yeasts, small round cells, sperms, mucus, and pathological casts (11 categories), as well as abnormal red blood cells. The particles that cannot be classified are reported as ''other cells.'' These alarms of problematic specimens have to be identified for manual microscopic urinalysis (14,15). It can analyze 100 samples in an hour. The UF-100 aspirates 800 μL of urine but requires a minimum sample volume of 4.0 mL and it has a manual system for emergency samples that only needs a volume of 1 mL. A large number of studies on this analyzer have been published (16–26).

The IRIS iQ200 analyzer uses digital imaging and Auto-Particle Recognition (APR$^{TM}$) (Chatsworth, CA) software to classify urine particles and quantitatively report results. Size, shape, and texture features are used by the APR software to classify each image into one of 13 categories: RBC, WBC, WBC clumps, hyaline casts, unclassified casts, EC, nonsquamous ECs, bacteria, yeast, crystals, mucus, sperm, and amorphous substances (27). Two paragraphs exist, which lack review alarms: the Summary where the images that do not strictly fulfill the classification criteria are stored and Microparticles (diameter lower than 15 μm), which includes the bacteria. The results obtained can be presented as units of cells/μL or units of cells/field (for RBC, WBC, EC, and bacteria), where the other particles are identified by flags. The IRIS iQ200 has a throughput of 60 samples/hr, requires a minimum sample volume of 3 mL, and only aspirates 2 μL. It has been evaluated (28,29) and compared with the manual study of sediments (30–33).

#### Manual microscopic sediment examination

The manual microscopic sediment examination was performed after samples were analyzed in the four systems considered. A procedure based on classical rules was established (34) and on those recommended by the NCCLS (35): 10 mL of urine sample were centrifuged at 2,000 rpm (450*g*) for 5 min according to the expression (36):

$$RCF(g) = 1.118 * 10^{-5} * r * (r.p.m.)^2 \qquad (1)$$

where RCF is the relative centrifugal force and $r$ the distance in cm between the rotation axis and the center of the sample tube.

After centrifugation, the pellet was carefully resuspended using an Eppendorf pipette. Then 20 µL was placed onto a microscope slide, which was covered with a slide cover measuring 20 × 20 mm. Several high power fields (HPFs) were assessed and the results were expressed as the mean of the count obtained per HPF. Then, the equivalence between the manual and automated sediment examination was

$$(Units\ of\ cells/\mu L) = (units\ of\ cells/field) * 5 \quad (2)$$

in agreement with the formula

$$Cell/\mu L = \frac{n * Vol_{pellet}}{\frac{Vol_{slide}}{HPF_{slide}} * Vol_{tube}} \quad (3)$$

where $n$ is the average number of cells per HPF, $Vol_{pellet}$ is the volume of the pellet after centrifugation (the average in our study was of 0.24 mL), $Vol_{slide}$ is the volume under the coverslip (20 µL), $HPF_{slide}$ is the ratio of the area of the slide and the area of one HPF $(20\,mm)^2/n(0.175\,mm)^2$, and $Vol_{tube}$is the total volume of urine in the test tube (10 mL) (35).

To reduce interobserver variability, the same two technologists performed all the microscopic urinalyses with the same microscope. The aim was to minimize the possible subjective trends inherent in the observer (systematical mistake for counting differences between both observers) that exceeds the random error based on Poisson's distribution (mistake inherent in the fact that particles are randomly distributed) (37). A double blind study was performed throughout.

The variables considered were those included in the strip tests, and the RBC, WBC, EC, bacteria, and crystals were measured by the quantitative automated urinalysis systems. Other sediment particles were not considered as they were present in a very small number of samples and statistically significant results could not be obtained for them.

The results have been categorized into three ranges and the dichotomic ones were classified as "negative" or "positive" (Table 1). The samples with values equal or up to 25 RBC or WBC/µL, positive nitrites, and or 10,000 bacteria/µL were considered as positive. The criteria used to classify the samples examined by a microscope as positive were five RBC or WBC/HPF and the presence of bacteria.

## Precision studies and carryover analysis

Within-run precision was determined by analyzing a pool of fresh specimens with various concentrations of RBC and WBC ten times each on the five analyzers considered. The between-run precision was determined over a 15-day period using a normal human lyophilized control containing stabilized human RBC and WBC particles at two different concentration levels (KOVA-Trol™, Hycor Biomedical (Garden Grove, CA), level I–pathologic and level III–normal). This control is useful for both automated urinary sediment analysis and qualitative procedures used in physicochemical determinations.

**TABLE 1. Result Ranges**

| Test | Procedure | Ranges 1 | 2 | 3 | Units |
|---|---|---|---|---|---|
| RBC/WBC | A, B | 0–25 | 26–150 | >150 | /µL |
| | C | 0–5 | 6–30 | >30 | /HPF |
| Density | B | 1.000–1.019 | 1.020–1.025 | >1.026 | g/L |
| pH | B | 5–5.5 | 6–7 | >7.5 | |
| Ketones | B | 0–5 | 15–50 | >80 | mg/dL |
| Glucose | B | 0 | 50–500 | >1,000 | mg/dL |
| Protein | B | 0 | 1–75 | >100 | mg/dL |
| Urubilinogen | B | *N* (<0.2) | *P* (>1) | | mg/dL |
| Bilirubin | B | *N* | *P* (>1) | | mg/dL |
| Nitrite | B | *N* | *P* | | |
| BAC | A | <10,000 | >10,000 | | /µL |
| | C | *N* | *P* | | |
| EC | A | 0–9 | 10–19 | ≥20 | /µL |
| | C | Low | Presence | High | /HPF |

RBC: erythrocytes; WBC: leukocytes; BAC: bacteria; EC: squamous epithelial cells; A: automated analyzers (UF-100 and iQ200); B: Dipstick readers (Atlas and Urisys); C: microscopy examination.

Carryover analysis was performed by analyzing a pool of negative specimens intercalated into other pathologic ones. The average value of five urine samples without the possibility of pollution ($Y$) was compared with that corresponding (average) to five negative urine samples tested immediately after the positive ones, that is, with a possibility of pollution ($X$):

*N*1-*N*2-*N*3-*P*1-*P*2-*N*4-*P*3-*P*4-*N*5-*N*6-*N*7-*N*8-*P*5-*P*6-*N*9-*P*7-*P*8-*N*10-*P*9-*P*10-*N*11

$$X = 1/5(N4 + N5 + N9 + N10 + N11)$$
$$Y = 1/5(N2 + N3 + N6 + N7 + N8).$$

## Workability studies of analyzers

The real throughput of the analyzers was estimated by assessing their starting times, alarms, and mistakes.

## Statistical analysis

To estimate the correlation between variables and to detect the presence of anomalous samples, a principal components analysis (PCA) was performed. This technique is used to reduce multidimensional *data sets*

to lower dimensions for analysis. PCA involves the computation of the *eigenvalue decomposition* or *singular value decomposition* of a data set, after mean centering and auto-scaling the data for each attribute (38). The results of a PCA are discussed in terms of scores and loadings. The relationship between the variables is described by the loading plot and the anomalous points are detected by influence plot. The Unscrambler 7.6 (CAMO ASA) (Trondheim, Norway) program was used.

Statistical analysis, including the nonparametric (variables considered do not fit a Gauss distribution) Wilcoxon test for measuring correlation and the McNemar test for measuring change in the distribution of two dichotomous variables, was performed by using SPSS 11.0 for Windows (Amsterdam, The Netherlands).

Spearman's coefficient was also calculated to determine the statistical level of signification ($P<0.01$). The *t*-Student test was applied to estimate imprecision to compare mean values ($P<0.05$: statistically significant differences).

## RESULTS

### Principal Components Analysis

As the variables considered are on different scales, data were auto-scaled before performing the PCA. A 20-principal components model was selected.

The loadings plot (corresponding to the two first principal components) showed a high positive correlation among the RBC, uric acid, and calcium oxalate crystals, and among WBC, nitrites/bacteria, triple phosphate, and amorphous phosphate crystals. On the other hand, an inversely proportional relationship was found between RBC and WBC and nitrite/bacteria.

Two anomalous points were detected from the influence plot, corresponding to the samples 282 and 426 for which the UF-100 analyzer generated a false alarm because of the presence of yeast (false-positive values). After eliminating these two samples from the model, the PCA was performed again, observing that the relationship found between the variables remained (no changes were observed in the influence plot), which revealed the robustness of the model (figures not shown).

### Concordance Level Between Automated and Manual Counts

Out of all the considered samples (652), the number of samples that were negative for the parameters studied by the five procedures studied (four analyzers and manual examination) was 294 (45%).

Taking into account the results obtained for the comparison between the different procedures by comparing two test strips analyzers, it can be concluded that the test strips analyzers showed a concordance level of more than 90% for all parameters considered, where the worst result obtained was that for the leukocytes (Table 2).

**TABLE 2. Atlas vs. Urisys Comparison**

| | Agreement | | |
|---|---|---|---|
| Parameter | *n* | % | Spearman's ρ |
| Erythrocytes | 587 | 91 | 0.676 |
| Leukocytes | 532 | 82 | 0.729 |
| Nitrite | 642 | 99 | 0.923 |
| Density | 560 | 86 | 0.861 |
| pH | 546 | 84 | 0.767 |
| Protein | 618 | 95 | 0.761 |
| Glucose | 638 | 99 | 0.955 |
| Ketones | 644 | 99 | 0.610 |
| Bilirubin | 644 | 99 | 0.532 |
| Urobilinogen | 629 | 97 | 0.356 |

From the comparison made between iQ200 and UF-100, a concordance level of approx. 75% was obtained for the RBC and WBC. The results obtained for the EC were very poor (Table 3).

When comparing the four analyzers with the manual sediment examination, the best level concordance was first obtained for nitrites/bacteria, followed by RBC, where the worst result was that obtained for WBC (Table 4).

The results for RBC (white area) and WBC (gray area) obtained for the comparison made between the five procedures considered are summarized in Table 5. Results are expressed as %: the central values correspond to the ties, that is, to the concordant results (in boldface); the top values represent those results that were higher in the recordings seen in the column than the recordings displayed in the row; low values correspond to those results that were lower in the recordings in the column than those shown in the row (for used ranges, see Table 1).

The best concordance level was obtained by the test strips analyzers for the RBC (91%), followed by the manual sediment examination (81–88%), iQ200 (80%), and UF-100 (70–74%), with counts as follows: from Atlas < Urisys ≤ manual sediment < iQ200 < UF-100.

Regarding WBC, the best concordance level was obtained for the test strips analyzers (82%). The concordance level of the remaining methods considered varied between 70 and 75%, with counts as followed: from Atlas = Urisys < iQ200 < manual sediment < UF-100.

The results obtained for RBC, WBC, and nitrites/bacteria were classified into five categories (see Table 6):

**TABLE 3. iQ200 vs. UF-100 Comparison**

| | Agreement | | |
|---|---|---|---|
| Parameter | *n* | % | Spearman's ρ |
| Erythrocytes | 477 | 75 | 0.506 |
| Leukocytes | 491 | 77 | 0.751 |
| Epithelial cells | 337 | 52 | 0.472 |
| Bacteria | 607 | 94 | 0.791 |
| Crystals | 608 | 95 | 0.170 |

**TABLE 4. Analyzers vs. Manual Examination of Sediment Comparison**

| | Erythrocytes | | | Leucocytes | | | Bacteria/Nitrites | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyzer | *n* | % | ρ | *n* | % | ρ | *n* | % | ρ |
| iQ200 | 523 | 81 | 0.473 | 475 | 73 | 0.695 | 564 | 87 | 0.538 |
| UF-100 | 473 | 74 | 0.439 | 498 | 78 | 0.761 | 584 | 91 | 0.647 |
| ATLAS | 568 | 88 | 0.525 | 447 | 69 | 0.684 | 577 | 89 | 0.532 |
| URISYS | 556 | 86 | 0.539 | 461 | 71 | 0.620 | 581 | 90 | 0.561 |

**TABLE 5. Results Obtained for the Comparison (% Concordance Degree) Made Between the Five Procedures Considered for Erythrocytes (White Area) and Leucocytes (Grey Area) Measurements**

| | Urysis | Atlas | Sediment | UF-100 | IQ200 |
|---|---|---|---|---|---|
| | | 9 | 23 | 26 | 16 |
| **Urysis** | | **82** | **71** | **71** | **74** |
| | | 9 | 5 | 3 | 9.5 |
| | 9 | | 25 | 26 | 16 |
| **Atlas** | **91** | | **69** | **70** | **75** |
| | 0 | | 5 | 3 | 9 |
| | 8 | 3 | | 13 | 7 |
| **Sediment** | **86** | **88** | | **78** | **73** |
| | 6 | 9 | | 9 | 20 |
| | 4 | 1 | 3 | | 3 |
| **UF-100** | **74** | **71** | **74** | | **76** |
| | 22 | 28 | 23 | | 20 |
| | 7 | 3 | 6 | 19 | |
| **IQ200** | **80** | **80** | **81** | **74** | |
| | 13 | 17 | 13 | 6 | |

See explanation in the text.

I, negative results for the five methods studied; II, positive results for the five methods; III, positive results for one of the methods and negative results for the rest, that is, false-positive values; IV, a negative result for a procedure as opposed to the positive result of the four remaining ones, that is, false-negative values; and V, two or more unlike, positive or negative results among the five procedures.

Other results not shown in Table 6 must be mentioned: the results obtained from the two test strips analyzers were negative but positive with the remaining methods (possible false negatives) for RBC in 10 samples (1.58%), for WBC in 32 (5.03%), and for nitrites/bacteria in 23 (3.62%). As for the automated urinalysis systems, they provided positive results that were negative for the rest of the procedures (possible false positive) in 33 samples (5.21%) for RBC, 16 (2.52%) for WBC, and 13 (2.04%) for nitrites/bacteria.

## Imprecision and Carryover

Within-run precision was slightly better for the UF-100 than for the iQ200 analyzer, and considerably better than for the manual sediment examination, which had a within-run coefficient of variation of more than 50% for all the studied parameters with low concentrations of cells.

Both test strips analyzers showed good precision except for the bilirubin parameter of the KOVA-Trol level I control measured in the Atlas analyzer. The results obtained for the within-run and between-run precision studies for UF-100, iQ200, and microscopy examination are summarized in Table 7.

Carryover studies showed that, in general, no substantial carryover was detected in any of the samples, demonstrating that the carryover was not systematic. Only a significant result for RBC measured in the iQ200 analyzer ($P = 0.046$) was found (Table 8).

## Workability Studies of the Analyzers

### Working times

Considering a pool of 50 samples, and including the starting time, the mean values were 13, 19, 46, and 35 min for the Atlas, Urisys, iQ200, and UF-100 analyzers, respectively.

The mean time needed for one sample was 19, 29, 77, 57, 94, and 164 sec for the Atlas, Urisys, iQ200, UF-100 analyzers, iQ200 screen revision, and manual sediment examination (also UF-100 revision), respectively.

### Errors

The Atlas analyzer attempted to aspirate liquid from empty positions on three occasions, generating an insufficient sample error.

No error from the Urisys 2400 analyzer throughout was observed.

The iQ200 did not process the samples with a clear color on seven occasions (detection errors). It also provided a communication error and a technical (microscopy) one, which led to having to initialize the instrument.

With regard to the UF-100 analyzer, a movement rack error was detected five times; it did not correctly read the bar code once; 11 samples were not correctly

**TABLE 6. Result Categories Obtained for Leukocytes (WBC), Erythrocytes (RBC), and Bacteria (BAC)/Nitrites**

| | | | | | | WBC | | RBC | | BAC/nitrite | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Atlas | Urisys | UF-100 | iQ200 | Manual | *n* | % | *n* | % | *n* | % |
| Category I | | | | | | | | | | | |
| All negatives | – | – | – | – | – | 295 | 46.38 | 362 | 57.10 | 487 | 76.57 |
| Category II | | | | | | | | | | | |
| All positives | + | + | + | + | + | 111 | 17.45 | 29 | 4.57 | 34 | 5.35 |
| Category III | – | – | – | – | + | 19 | (2.99) | 9 | (1.42) | 30 | (4.72) |
| False positives | – | – | – | + | – | 13 | (2.04) | 30 | (4.73) | 23 | (3.62) |
| | – | – | + | – | – | 25 | (3.93) | 77 | (12.15) | 7 | (1.10) |
| | – | + | – | – | – | 2 | (0.31) | 13 | (2.05) | 0 | (0.00) |
| | + | – | – | – | – | 6 | (0.94) | 0 | (0.00) | 0 | (0.00) |
| Total | | | | | | 65 | 10.21 | 129 | 20.34 | 60 | 9.50 |
| Category IV | + | + | + | + | – | 4 | (0.63) | 4 | (0.63) | 5 | (0.79) |
| False negatives | + | + | + | – | + | 10 | (1.57) | 5 | (0.79) | 1 | (0.16) |
| | + | + | – | + | + | 0 | (0.00) | 2 | (0.32) | 0 | (0.00) |
| | + | – | + | + | + | 26 | (4.09) | 1 | (0.16) | 1 | (0.16) |
| | – | + | + | + | + | 8 | (1.26) | 16 | (2.52) | 4 | (0.63) |
| Total | | | | | | 48 | 7.54 | 28 | 4.41 | 11 | 1.72 |
| Category V | | | | | | | | | | | |
| Remain | | | | | | 117 | 18.39 | 86 | 13.56 | 39 | 6.1 |
| Lost | | | | | | 16 | | 18 | | 15 | |
| Total | | | | | | 652 | | 652 | | 652 | |

**TABLE 7. Within-Run (A) and Between-Run (B) Precision Studies**

| | UF-100 | | iQ200 | | Microscopy examination | |
|---|---|---|---|---|---|---|
| | Rank (/μL) | CV (%) | Rank (/μL) | CV (%) | Rank (/μL) | CV (%) |
| (A) | | | | | | |
| RBC | 4–15 | 37.3 | 0–9 | 45.5 | 1–17 | 62.3 |
| | 310–430 | 10.6 | 170–251 | 12.5 | 190–360 | 22.0 |
| | 791–921 | 5.0 | 680–791 | 4.8 | 575–1,150 | 21.4 |
| WBC | 7–20 | 23.1 | 6–18 | 34.1 | 4–24 | 57.7 |
| | 99–150 | 14.3 | 75–120 | 13.0 | 55–150 | 25.4 |
| | 550–701 | 6.7 | 495–601 | 6.8 | 450–850 | 17.8 |
| (B) | | | | | | |
| RBC | 3–15 | 39.6 | 1–8 | 46.8 | | |
| | 50–99 | 20.5 | 31–74 | 21.2 | | |
| WBC | 20–39 | 21.6 | 23–48 | 24.7 | | |
| | 72–140 | 19.4 | 99–181 | 17.6 | | |

RBC: erythrocytes; WBC: leukocytes; CV: coefficient of variation.

**TABLE 8. Carryover Studies**

| RBC | UF-100 | iQ200 | WBC | UF-100 | iQ200 |
|---|---|---|---|---|---|
| *X*–*Y* | 2.2 | 6 | *X*–*Y* | 0.2 | 1.8 |
| SD | 2.77 | 4.69 | *SD* | 2.77 | 5.40 |
| *t* | 1.77 | 2.86 | *t* | 0.16 | 0.74 |
| *P* | 0.151 | 0.046 | *P* | 0.880 | 0.498 |

RBC: erythrocytes; WBC: leukocytes; *X*: samples with the possibility of pollution; *Y*: samples without the possibility of pollution.

analyzed at the first time, and a second attempt was made in two of them owing to turbidity reasons, which meant that the manual sediment examination necessary.

## Reviews

Taking into account the results provided by the iQ200, a manual sediment revision of 97 samples was needed (14.8% of the total). The sum of 125 alarms was generated mainly owing to amorphous characteristics of particles (66%), followed by crystals (33%) and yeast (27%). After revision, 104 discordant results were found, which, once again, were because of amorphous characteristics of particles (33%) but also because of bacteria (24%) and yeast (16%). Comparing with the manual sediment examination, 113 discordant results were found for RBC (23%), WBC (15%), bacteria (15%), amorphous characteristics of particles (15%), and EC (14%). It is worth indicating that the more frequently generated alarm, the amorphous characteristics of particles, was demonstrated to be true in a third of the cases. Another third was actually because of bacteria.

The UF-100 system generated 259 alarms for 134 samples, mainly because of RBC (40%), followed by

WBC (22%), bacteria (20%), and EC (13%). In comparison with the manual sediment examination, the discordant results found were also owing to RBC (29%), EC (25%), bacteria (21%), WBC (13%), and crystals (9%). It is important to indicate that 23 of the 80 elevated false results for RBC were because of the presence of crystals.

## DISCUSSION

Although there is no established reference method for urinalysis, the manual analysis of urine sediment continues to be considered as the suitable method for routine laboratories (10). Nevertheless, it is fraught with methodological problems, which generate high imprecision when compared with automated methods (5). A substantial amount of different factors may contribute to the imprecision in manual urinalysis (3). For example, they may range from incomplete pelleting of the sediment, cellular lysis because of centrifugation, or from problems when the sediment is resuspended, which lead to different interpretations by different technologists of a cell or cast in a urine sediment (39). Good staff training is also important (32). Moreover the process is time-consuming, a very important factor to consider in laboratories with a large volume of work.

In this study, in contrast to other published ones, the results obtained for the main urine elements studied have been compared one by one, working with the samples in an independent manner using five different methods; among them the microscopy manual examination was considered as another method rather than a tool for discrepancies to be solved. In addition, the fact that we had a statistical method such as the PCA, which allowed us to detect anomalous samples in a previous phase of the study, made their exclusion possible from the later statistical analysis, this being more reliable.

In general, for the main urine parameters evaluated an adequate concordance degree, up to 69%, was found between all the methods considered. These data were similar to others reported (2,12,32). For the main anomalous urine elements, a high percentage of the samples were correctly identified by means of the five analytical methods evaluated (64% for WBC, 62% for RBC, and 82% for BAC/nitrites). A low number of false-positive and false-negative results were found, except for the RBC counting by the UF-100 analyzer for which a variation coefficient about 12% was obtained but similar to that reported in other published studies (21,40). It was also observed that, despite the low number of false-positive and false-negative results provided by the test strips analyzers, the highest false-negative results for WBC (esterases) were obtained with the Urisys system. This one also provided higher false-positive results for RBC, whereas the Atlas analyzer provided the highest false-negative results for the same parameter.

In this study, both test strips analyzers showed good features and similar throughputs, as reported in previous studies (40), although the Atlas model was slightly faster. This model generated different errors because of changes in the sampler to racks in order to achieve the best integration to other analyzers and to make the samples transfer faster from one analyzer to another. Nevertheless, there is a need for a more quantitative evaluation of urinalysis test strips, because they are used for checking automated urine analyzers.

The UF-100 has two important advantages: offers the better precision for low units of cells when compared with the iQ200 and it is the faster analyzer, in spite of the difference observed in our study being shorter (UF-100: 86 samples/hr, iQ200: 65 samples/hr) than the one specified by the manufacturer. However, it required a large number of manual sediment revisions and the RBC counting showed false high values because of the presence of crystals. Another advantage of the UF-100 analyzer is the scattergram; it provides more precise information about red blood cell morphology (15,17). In general, the results obtained for both RBC and WBC were higher than those obtained for the same two parameters with the other procedures considered, which has been also observed in other published works (2,9,18,21,39). Another relevant aspect of this analyzer is the set for urgent sample examination, which requires a shorter sample volume making the analysis of limited samples such as the pediatric ones easier.

One of the principal iQ200 advantages is the lowest number of revisions required and the possibility of performing these revisions on the instrument screen without the need of a manual sediment examination. However, it is necessary to undergo major staff training so that the staff become used to identifying the images shown (41), which differ substantially from the manual observation.

Unlike the UF-100 analyzer, the iQ200 does not generate an alarm, either for the revision when RBC and WBC values are more than 9,000 units of cells/L (this situation was observed only twice throughout our study) or for the nonclassified samples that must be recovered by the technologist so that they are assessed. In addition, another restriction that the iQ200 analyzer has is that it does not generate an alarm for the presence of bacteria (32). In our study, 33% of all samples that were classified as amorphous particles presented bacteria.

On the whole, precision studies provided adequate variation coefficient values except for the samples with poor cellularity, as observed in other published studies

(9,18,30,39). The imprecision was slightly higher for the iQ200 analyzer. From the carryover studies, it is concluded that no significant increase in the mean values of the negative pool samples following the pathological urine samples ($P > 0.05$) was found, except for WBC measured by the iQ200 analyzer as was mentioned before.

In comparison with the manual sediment examination, the UF-100 overestimates cell counts, especially that corresponding to RBC, whereas the iQ200 system does not show this tendency.

The differences observed between the results obtained for RBC and WBC with the strip test analyzers with those obtained with the other procedures considered could be owing to the different methodology used to determine these parameters: the strips test analyzers measure enzymatic activity and do not count cell units. On the other hand, there can be lecture interferences in these types of analyzers because of urine with high values of SG, glucose, protein, chemicals (ascorbic acid, cephalosporin, penicillin, and derivatives), etc.

What we conclude from this study is that either of the two test strips analyzers that adapt to an automated urinalysis system provides clinically acceptable results, which improve as far as precision and efficiency are concerned. And related to the iQ200, it would be ideal that the software required for identification of bacteria was included in a new version of this instrument (IRIS iQ200 Sprint, Chatsworth, CA).

As already mentioned, both systems (Sysmex UF-100 and IRIS iQ200) can discriminate pathologic samples from the nonpathologic ones, depending on certain user-established criteria to allow for a self-validation system so that only those samples that have generated alarms are checked.

To guarantee the quality of the results sent to clinical laboratories, one essential condition is the creation of a few filters that process the received information from both systems (test strips and automated urinalysis analyzers) to avoid incongruent results.

Taking into account that sample turbidity, color, or abundant cellularity can influence the results provided by the analyzers, it would be interesting to supply a mechanism for sample dilution as another technical feature.

It would also be very interesting to perform other studies with other available test strips analyzers not considered in this work.

## ACKNOWLEDGMENTS

The authors acknowledge Bayer Diagnostics, Izasa S.A., and Roche Diagnostics for their technical support. The last two companies provided the reagents needed to carry out the study.

## REFERENCES

1. Winkel P, Statland BB, Jergensen K. Urine microscopy, and III-defined method, examined by a multifactorial technique. Clin Chem 1974;20/4:436–439.
2. Langlois MR, Delanghe JR, Steyaert SR, Everaert KC, Buyzere ML. Automated flow cytometry compared with an automated dipstick reader for urinalysis. Clin Chem 1999;45:118–122.
3. Carlson DA, Statland BE. Automated urinalysis. Clin Lab Med 1988;8:449–461.
4. Wargotz ES, Hyde JE, Karcher DS, Hitlan JP, Wilkinson DS. Urine sediment analysis by the Yellow IRIS automated urinalysis workstation. Am J Clin Pathol 1987;88:746–748.
5. Deindorfler FH, Gangwer JR, Laird CW, Ringold RR. The Yellow IRIS urinalysis workstation. The first commercial application of automated intelligent microscopy. Clin Chem 1985;31: 1491–1499.
6. Gai M, Piccoli GB, Segoloni GP, Lanfranco G. Microscopic urinalysis and automated flow cytometry in a nephrology laboratory. Clin Chem 2003;49:1559–1560.
7. Steinmetz J, Henry J, Gueguen R. Etablissement de limites de reference des sediments urinaires measures sur UF-50TM Sysmex. Ann Biol Clin 2004;62:671–680.
8. Solomon R, Heller K, Gutman S. Clinical significance of dipstick negative, sediment positive urines: Two reagent dipsticks compared. [Letter] Clin Chem 1987;33:418.
9. Hannemann-Pohl KH, Kampf SC. Automation of urine sediment examination: A comparison of the Sysmex UF-100 automated flow cytometer with routine manual diagnosis (microscopy, test strips, and bacterial culture). Clin Chem Lab Med 1999; 37:753–764.
10. Kouri TT, Gant VA, Fogazzi GB, Hofmann W, Hallander HO, Guder WG. Towards European urinalysis guidelines. Introduction of a project under European confederation of laboratory medicine. Clin Chim Acta 2000;297:305–311.
11. European Urinalysis Group. European urinalysis guidelines. Scan J Clin Lab Invest 2000;60:1–96.
12. Penders J, Fiers T, Delangue JR. Quantitative evaluation of urinalysis test strip. Clin Chem 2002;48:2236–2241.
13. Dias VC, Moschopedis T, Prosser C, Yatscoff RW. Evaluation of the Clinitek® AtlasTM for routine macroscopic urinalysis. Clin Biochem 1996;29:217–223.
14. Delangue JR, Kouri TT, Huber AR, et al. The role of automated urine particle flow cytometry in clinical practice. Clin Chim Acta 2000;301:1–18.
15. Chan RW, Szeto CC. Advances in the clinical laboratory assessment of urinary sediment. Clin Chim Acta 2004;340:67–78.
16. IUC Product Development Division TOA Medical Electronics. Performance of the Sysmex UF-100 fully automated urine cell analyzer. Sysmex J Int 1996;6:36–40.
17. Apeland T, Mestad O, Hetland O. Assessment of haematuria: Automated urine flowmetry vs microscopy. Nephrol Dial Transplant 2001;16:1615–1619.
18. Ben-Ezra J, Bork L, McPherson RA. Evaluation of the Sysmex UF-100 automated urinalysis analyzer. Clin Chem 1998;44:92–95.
19. Fenili D, Pirovano B. The automation of sediment urinalysis using a new urine flow cytometer (UF-100). Clin Chem Lab Med 1998;36:909–917.
20. Kouri TT, Kahkonen U, Malminiemi K, Vuento R, Rowan RM. Evaluation of Sysmex UF-100 urine flow cytometer vs chamber

counting of supravitally stined specimens and conventional bacterial cultures. Am J Clin Pathol 1999;112:25–35.
21. Ottiger C, Huber AR. Quantitative urine particle analysis: Integrative approach for the optimal combination of automation with UF-100 and microscopic review with KOVA cell chamber. Clin Chem 2003;49:617–623.
22. Manoni F, Valverde S, Antico F, Salvadego MM, Gacomini A, Gessoni G. Field evaluation of a second-generation cytometer UF-100 in diagnosis of acute urinary tract infections in adult patients. Clin Microbiol Infect 2002;8:662–668.
23. Dimech W, Roney K. Evaluation of an automated urinalysis system for testing urine chemistry, microscopy and culture. Pathology 2002;34:170–177.
24. Roggeman S, Zaman Z. Safely reducing manual urine microscopy analyses by combining urine flow cytometer and strip results. Am J Clin Pathol 2001;116:872–878.
25. Regeniter A, Haenni V, Risch L, et al. Urine analysis performed by flow cytometry: Reference range determination and comparison to morphological finding, dipstick chemistry and bacterial culture results—A multicenter study. Clin Nephrol 2001;55:384–392.
26. Lun A, Ziebig R, Priem F, Piller G, Sinha P. Routine workflow for use of urine strips and urine flow cytometer UF-100 in the hospital laboratory. Clin Chem 1999;45:1305–1307.
27. Haliassos A, Katritsis D, Mandalaki E, Bizioura L, Antonopoulou R, Chiotinis N. Evaluation of integration of the Iris diagnostics iQ200TM automated urine microscopy analyzer in a clinical laboratory. Clin Chem 2005;51:A214–A215.
28. Lee G, Lee K, Yoon S, et al. Evaluation of the iQ200 automated urine microscopy analyzer. [Abstract] Clin Chem 2005;51:A216.
29. Cappelleti P, De Rosa R, Mottola A, Biasioli B. Evaluation of the iQ200 automated urine microscopy analyzer. [Abstract] Clin Chem 2005;51:A221.
30. Wah DT, Wises PK, Butch AW. Analytic performance of the iQ200 automated urine microscopy analyzer and comparison with manual counts using Fuchs–Rosenthal cell chambers. Am J Clin Pathol 2005;123:290–296.
31. Lamchiagdhase P, Preechaborisudkul K, Lomsomboon P, et al. Urine sediment examination: A comparison between the manual method and the iQ200 automated urine microscopy analyzer. Clin Chim Acta 2005;358:167–174.
32. Alves L, Ballester F, Camps J, Joven J. Preliminary evaluation of the Iris iQ200 automated urine analyzer. Clin Chem Lab Med 2005;43:967–970.
33. Vogiatzakis E, Kouvardas S, Gkoka A, et al. Comparison and evaluation of urine particle analysis methods: The iQ200 automated urine microscopy analyzer vs. manual microscopy. Clin Chem 2005;51:A215–A216.
34. Fuller E, Threalte GA, Henry JB. 2005. Examen básico de la orina. In: JB Henry, editor. El Laboratorio en el Diagnóstico Clínico. ed original. Madrid: Marban Libros.
35. National Committee for Clinical Laboratory Standards. Urinalysis and collection, transportation and preservation of urine specimens; approved guideline (NCCLS document GP16-A). Villanova PA: NCCLS, 1995. p 51–69.
36. Henry JB, Kurec AS. 2005. Laboratorio clínico: Organización, objetivos y práctica. In: JB Henry, editor. El Laboratorio en el Diagnóstico Clínico. ed original. Madrid: Marban Libros.
37. Kouri T, Gyory A, Rowan RM. ISLH recommended reference procedure for the enumeration of particles in urine. Lab Haematol 2003;9:58–63.
38. Wold S, Esbensen K, Geladi P. 1987. Principal Component Analysis. Chimometrics and Intelligent Laboratory Systems. Amsterdam: Elsevier Science Publishers, B.V.
39. Chien TI, Kao JT, Liu HL, et al. Urine sediment examination: A comparison of automated urinalysis systems and manual microscopy. Clin Chim Acta 2007;384:28–34.
40. Tofaletti J, Dotson MA, Shearman P, Koontz A. Comparison of two automated systems for urine chemistry and urine sediment analysis. Lab Haematol 1999;5:123–129.
41. Linko S, Kouri TT, Toivonen E, Ranta PH, Chapoulaud E, Lalla M. Analytical performance of the Iris iQ200 automated urine microscopy analyzer. Clin Chim Acta 2006;372:54–64.