Quantitative Evaluation of Urinalysis Test Strips

Joris Penders, Tom Fiers, and Joris R. Delanghe*  

Background: Urine test strip results are generally reported in categories (i.e., ordinal scaled), but automated strip readers are now available that can report quantitative data. We investigated the possible use of these meters to complement flow cytometry of urine and compared reflectance readings with quantitative determinations of urinary glucose and microalbumin.

Methods: We compared URISYS 2400 (Roche) quantitative reflectance data with data from the UF-100 (Sysmex) and biochemical data for 436 nonpathologic and pathologic urine samples.

Results: Reproducibility of the reflectance signal was good for high- and low-concentration urine pools for protein (0.8% and 0.9% and 1.5% and 2.2% within and between runs, respectively), leukocyte esterase (1.1% and 1.0%; 5.1% and 1.2%), hemoglobin (1.7% and 1.1%; 8.9% and 1.1%) and glucose (2.1% and 0.5%; 6.5% and 2.3%). Fair agreement was obtained between UF-100 and test strip reflectance data for erythrocytes and hemoglobin (r = −0.680) and leukocytes and leukocyte esterase (r = −0.688). Higher correlations were observed for biochemical and test strip data comparing protein and albumin (r = −0.825) and glucose data (r = −0.851). The lower limits of detection for erythrocytes and leukocytes were 8 × 10⁶/L and 19 × 10⁶/L, respectively. The protein test (n = 220) detected 86% (95% confidence interval, 78–92%) of samples with <30 mg/L albumin with a specificity of 84% (95% confidence interval, 76–91%).

Conclusions: In urine test strip analysis, quantitative hemoglobin and leukocyte esterase reflectance data are complementary with flow cytometric results and glucose and albumin results.

Test strip analysis plays an important role in urinalysis as such, and the value of test strip urinalysis as a screening method has been thoroughly demonstrated (1). The reproducibility of (semi)automated readings is at least as good as visual readings (2), but most authors find the analytical, clinical, and labor cost-saving advantages of (semi)automated vs visual reading to be obvious (3).

Recently, the URISYS 2400 automated urine test strip analyzer (Roche Diagnostics) was introduced. This instrument offers the possibility to obtain reflectance readings. Test results, therefore, no longer need to be expressed in an ordinal scale. Access to the instrument’s raw data theoretically allows a higher analytical sensitivity for several analytes. Because microalbuminuria is generally regarded as an excellent marker for assessing early renal damage in common conditions such as diabetes and hypertension (4–7) and as an early predictor of pre-eclampsia during pregnancy (8), the availability of highly sensitive test strip readers opens interesting perspectives for assessing this phenomenon.

Flow cytometry has been introduced for urinalysis (9, 10) to obtain quantitative data on urinary particles. The imprecision of urinary flow cytometry is far less than that of conventional urinary microscopy, but in some cases urinary flow cytometry reports erroneous results because of analytical interferences (e.g., calcium carbonate crystals falsely increase erythrocyte counts; confusion between yeast cells and erythrocytes). Langlois et al. (11) reported disagreement in erythrocyte counts between the UF-100 and the hemoglobin test strip reaction in 6.5% of cases. Of course, the frequency of erroneous results depends on the proportion of pathologic samples and on the preanalytical handling of samples. Combining diagnostic information provided by urinary flow cytometry and more quantitative test strip analysis therefore offers a theoretical basis for the development of diagnostic expert systems (11, 12).

In this study, we wanted to investigate the performance of quantitative urinary test strip analysis. In particular, we wanted to compare the reflectance readings of the protein test field with immunochemical microalbumin determinations. We also wanted to explore the possibilities of combining the two novel methods, particularly in the analysis of erythrocytes, leukocytes, and glucose, which are of major clinical importance.
**Materials and Methods**

**Patients and samples**

We studied 436 freshly collected urine samples submitted to our routine laboratory for diagnostic urinalysis. All samples were completely processed within 2–4 h after arrival. Test strip urinalysis was carried out before flow cytometry analysis (Sysmex UF-100; TOA Medical Electronics), using URISYS strips on a URISYS 2400 analyzer (Roche Diagnostics). Combaur 10-Test M strips on a Miditron automated reflectance photometer (Roche) (13, 14), used in our routine laboratory, were used in parallel as a control. The strips include reagent pads for ordinal scale reporting of relative density, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and hemoglobin/myoglobin.

Day-to-day imprecision was assessed with control material: Liquichek Urinalysis Control Levels 1 and 2 (Bio-Rad). This is stable for 30 days when stored tightly capped at 2–8 °C.

**Urinary Flow Cytometry**

The Sysmex UF-100 is a urinary flow cytometer-based walkaway instrument that performs automated microscopic analysis. It has been extensively evaluated for urinalysis (9–11, 15) as well as for cerebrospinal fluid analysis (16). The principle is based on argon laser flow cytometry. The UF-100 measures the conductivity and categorizes the particles on the basis of their shape, size, volume, and staining characteristics. The results are displayed in scattergrams, histograms, and as counts per microliter as well as counts per high-power field. The UF-100 automatically detects and counts red blood cells (RBCs), white blood cells (WBCs), bacteria, yeast cells, crystals, epithelial cells, small round cells, sperm cells, and casts. Particles that cannot be classified in one of the former categories are counted as “other cells”.

**URISYS 2400**

Urine test strip analysis was performed with the automated URISYS 2400. These test strips are the same as used in the Miditron but are supplied in a cassette holding 400 test strips for leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, hemoglobin/myoglobin, and pH.

The intensity of the reaction color of the test pad is detected by measuring the percentage of light reflected from the surface of the test pad. The higher the analyte, the higher the color intensity and, thus, the lower the reflectance. The reflectance value, expressed as a percentage within a range from 100% (white) to 0% (black), is therefore inversely related to the concentration of the analyte in the sample. Specific gravity (refractometry based) and clarity are measured in a flow cell, and color is rated with a specific algorithm against the blank pad on the test strip. Data are expressed in an ordinal scale (as “normal”, “negative”, “positive”, or as nominal concentrations) on the reports, but (quantitative) reflectance data can be downloaded to floppy disks.

**Biochemical Investigations**

Albumin in urine was measured immunonephelometrically on 220 randomly selected samples with use of commercially available Behring antibodies on a Behring Nephelometer II analyzer (Dade Behring) standardized against the widely accepted WHO/College of American Pathologists Certified Reference Material 470. Glucose was measured by a hexokinase method standardized against Standard Reference Material 917a and Standard Reference Material 965 (n = 85), and total protein by a pyrogallol red method (17) (n = 129) with Standard Reference Material 917a as a standard and commercially available reagents for both measurement procedures (Roche) on a Modular P system (Roche).

**Statistics**

P values <0.05 were considered significant. Agreement between automated flow cytometry and test strip data was evaluated by Spearman rank analysis. Multiple regression analysis was used to investigate a model relating leukocyte esterase and hemoglobin field reflectance. The lower limit of detection (18) was calculated as the mean value – 3 SD for a blank sample. Diagnostic accuracy was assessed by ROC analysis using commercially available statistical software (MedCalc®).

**Results**

**Dilution and pH**

Alkalization of urine gives rise to erroneous results in particle counting. When monitoring our samples, we found a median pH of 6.5 (95% interval, 5–8). Hence, extremely alkaline urine samples were not present in the samples we investigated. Dilution was also monitored with a range of specific gravity of 1.005–1.033 and a median result of 1.017.

**Reproducibility**

The within- and between-run CVs for protein, leukocyte esterase, hemoglobin, and glucose are summarized in Table 1.

**Comparison of Protein Reflectance Results, Albumin, and Proteinuria**

We found a close correlation between the log-transformed albumin results (x) and the test strip reflectance results (y): y (%) = 68.2 – 9.26[logx (mg/L)]; Spearman r = –0.825; P <0.001; Fig. 1. Two discrepancies (0.9%) were found in which the test strips on both automated strip readers overestimated the urinary albumin concentration by >10-fold.

Similarly, protein reflectance data and total protein measurements correlated well (Spearman r = –0.921; P <0.001), which is expected as long as the major protein is albumin. The lower limit of detection was 25 mg/L (CV =
On the basis of ROC analysis, diagnostic sensitivity was 86% (95% confidence interval, 78–92%) and specificity was 84% (95% confidence interval, 76–91%) for a reflectance value of 55.6% when the nephelometric assay was used as the comparison method at a cutoff of 30 mg/L (n = 220; 113 positive and 107 negative cases, respectively).

**Comparison of Flow Cytometric RBC and Hemoglobin Reflectance Results**

Agreement was fair between the flow cytometric RBC data (x) and the URISYS 2400 hemoglobin reflectance measurements (y) for counts above the upper reference limit (25 × 10⁶ RBC/L on UF-100). The following regression equation was obtained: y (%) = 78.2 – 19.4 logx (10⁶ cells/L); Spearman r = –0.680; P < 0.001; Fig. 2. A lower limit of detection of 8 × 10⁶ cells/L (CV = 1.2%) was calculated.

### Table 1. Reproducibility of protein, leukocyte esterase, hemoglobin, and glucose on the URISYS 2400 analyzer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein (albumin)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean reflectance, %</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>CV, %</td>
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<td>2.2</td>
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<tr>
<td><strong>Leukocyte esterase</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean reflectance, %</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>CV, %</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
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<td></td>
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<tr>
<td>Mean reflectance, %</td>
<td>52.0</td>
<td>38.3</td>
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<tr>
<td>CV, %</td>
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<td>1.5</td>
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<tr>
<td><strong>Glucose</strong></td>
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<tr>
<td>Mean reflectance, %</td>
<td>62.0</td>
<td>60.2</td>
</tr>
<tr>
<td>CV, %</td>
<td>1.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**Fig. 1.** Correlation between protein (albumin) results obtained by quantitative test strip analysis (y) and albumin (x; Behring BN II Nephelometer; n = 220).

Protein field reflectance (y; %) = 68.2 – 9.26 log(albumin; mg/L); Spearman r = –0.825; P < 0.001. The two outer solid lines represent the 95% prediction interval around the regression line. The dashed lines represent the lower limit of detection (— — —) with the 2 SD limits (— — —).

**Fig. 2.** Correlation between RBC counts (x; flow cytometry) and test strip hemoglobin concentration (y) for RBC counts >25 × 10⁶ cells/L (n = 96).

Hemoglobin reflectance (y; %) = 78.2 – 19.4 log(RBC count; 10⁶ cells/L); (Spearman r = –0.680; P < 0.001. The two outer solid lines represent the 95% prediction interval around the regression line. The dashed lines represent the lower limit of detection (— — —) with the 2 SD limits (— — —).
Because RBCs tend to lyse in urine, we investigated the linearity of the hemoglobin test strip pad. The UF-100 flow cytometer automatically determines the conductivity, so we performed multiple regression analysis on the study population, calculated in a regression model the hemoglobin reflectance vs RBCs and conductivity, and found that the t-value (as expected) for RBCs was −8.442 (P <0.0001). In contrast, conductivity showed a t-value of only 1.477 (P = 0.141). Additional dilution experiments were carried out. The ratio of hemoglobin reflectance to RBC count was constant when osmolality was >190 mosmol/L.

Figure 3. Regression equation for urinary WBC counts above the upper reference limit (25 × 10⁶ cells/L; n = 132).

Leukocyte esterase reflectance (y %) = 83.7 −15.4 log (WBC count; 10⁶ cells/L); Spearman r = −0.688; P <0.001. The two outer solid lines represent the 95% prediction interval around the regression line. The dashed lines represent the lower limit of detection (−−−−) with the 2 SD limits (−−−−).

Discussion

The change from urine microscopy to urinary flow cytom-etry has been accompanied by a significant decrease in imprecision (15). Because urinalysis test strips are often used for checking urinary flow cytometry data (11), there is a need for a more quantitative evaluation of urinalysis test strips. In this study, we compared the URISYS 2400 automated strip reader with the Sysmex UF-100 flow cytometer to evaluate the possible value of quantitative test strip data in a urinary expert system.

The turnaround time was short enough (2–4 h) not to affect the readings: time intervals can influence results of automated test strip analysis, especially leukocyte and erythrocyte ratings (19).

The detection limit of the URISYS 2400 protein assay was 25 mg/L if restricted to albumin alone and total protein was not considered. Because microalbuminuria is defined as excretion of 30–300 mg of albumin/24 h (20–200 µg/min, or 30–300 µg/mg of creatinine) (20), the protein field result allows identification of microalbuminuria cases in contrast to the classic reading and reporting of the strips, which can detect only albumin concentra-tions 150–200 mg/L or higher (21, 22). This implies that the test has the potential to offer a screening for microalbuminuria without increased cost, hence coming closer to the “urine test strip of the future” (23). This could improve level 1 urinary screening (12); it offers an improved, fast, reliable method that is easy to handle and usable in primary-care laboratories. It could be of special interest in patients with undiagnosed diabetes or hypertension in whom microalbuminuria is regarded as an excellent marker for assessing early renal damage (4–7), particularly because many patients with non-insulin-de-pendent diabetes mellitus are asymptomatic and their diabetic state remains undiagnosed for years (24). It must be noted, however, that the sensitivity (86%) may be inadequate for patients with known diabetes in whom physicians do not want to miss microalbuminuria in their annual testing. Moreover, a 16% false-positive rate may be unacceptable for screening.

Because microalbuminuria is characterized by large intra-individual variability, an ordinal-scale answer might...
be misleading. It therefore is advantageous to have a better estimation of the true concentration.

As is the case for test strips in general, the method is based on the so-called “indicator error” principle, in which proton exchange between the indicator on the strip and the proteins in the solution produces a color change from yellow to green-blue. Of all the diagnostically relevant urinary markers, only albumin and transferrin accept protons well, so the potential error of missing Bence Jones proteinuria remains when test strip screening strategies are used in proteinuria (21), although many cases presenting with Bence Jones or tubular proteinuria show microalbuminuria (23).

In recent studies, the correlation (r) between UFC WBCs and WBCs by counting chamber was 0.93–0.98, and that of UFC RBCs and RBCs by counting chamber was 0.83–0.89 (10, 25, 26). In our study correlating test strip data with urinary flow results, r was −0.69 between the flow cytometric WBC count and the leukocyte esterase reaction, although the presence of esterase inhibitors in urine and severe proteinuria might negatively affect test results for leukocyte esterase (1, 27). No effect of conductivity on the leukocyte esterase field was noted. Test strip measurements had reasonable lower limits of detection for WBC (19 × 10⁶/L).

In this study, the urinary hemoglobin concentration did not agree well with the RBC count obtained by flow cytometry. The hemoglobin measurement is based on the peroxidase principle. It is known that reducing substances (e.g., ascorbic acid) may lower the signal, whereas oxidizing substances may have a positive effect on measured hemoglobin concentration. Various low- and high-molecular mass inhibitors have been found in urine (28, 29). The presence of haptoglobin in urine enhances the peroxidase activity of hemoglobin (29, 30). Bacterial peroxidases can also contribute to total peroxidase activity in urine (23). On the other hand, the quantitative evaluation of test strips may help to eliminate analytical errors in RBC counting attributable to the presence of yeast cells or large amounts of calcium carbonate crystals (11, 15). As shown by our multiple regression model and dilution experiments, the hemoglobin field reflectance test is not influenced by dilution effects. As is the case for WBCs, the lower limit of detection for RBCs is acceptable (8 × 10⁶/L).

The correlation coefficient for the glucose signal with the routine hexokinase-based method was −0.851. The presence of ascorbate oxidase on the glucose test field prevents interference by ascorbic acid.

Because of the procedure for applying the urine to the test strips on the URISYS 2400, improper dipping is no longer a problem, nor is confusion about sample identification or urine contamination caused by dipping the strip in the tube, which potentially leads to interferences with chromatographic methods (31).

In conclusion, quantitative urine test strip analysis provides reliable data on WBCs, RBCs, glucose, and albumin. This offers several possibilities: (a) The sensitivity for albumin may allow affordable screening for microalbuminuria, particularly in patients with undiagnosed renal damage. To fully explore the possibilities of the albumin test pad in first-line diagnosis, a formal study should be performed. (b) In addition, hemoglobin and leukocyte esterase reflectance data are useful for verifying flow cytometric data on RBCs and WBCs. This leads to improved elimination of occasional errors in the WBC and RBC counting channels of the flow cytometer (11).

**References**


