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Routine Workflow for Use of Urine Strips and Urine Flow Cytometer UF-100 in the Hospital Laboratory,[1]

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None of the methods currently available for evaluating hematuria and leukocyturia is perfect. Urinalysis by test strip combined with automated particle counting is an attractive approach that may identify false-negative results of both techniques. We wished to evaluate the possibility that microscopic evaluation of urine samples could be substantially reduced by this approach.

The fully automated urine flow cytometer UF-100 classifies urinary particles on the basis of their light scattering, fluorescence, and impedance properties. The instrument counts erythrocytes, leukocytes, bacteria, epithelial cells, and casts and flags the presence of pathological casts, small round cells (SRCs), yeast-like cells, crystals, and spermatozoa. The instrument is intended to replace, to an extent, routine urine microscopy. The operating principles of the UF-100 *(1)*; its precision, accuracy, and analytical sensitivity *(2)*; and its potential for differentiation between renal and postrenal hematuria *(3)* have been published previously *(4)*.

The precision of particle counting in microscope chambers is poorer than counting by flow cytometry *(5)*. The analysis of urine samples by UF-100 and the test strip analyzer Clinitek Atlas showed discordant results in a small but not negligible number of cases. This result has also been described by Ben-Ezra et al. *(4)*. We therefore rechecked the false results in erythrocyte and leukocyte counts by examining discrepant results from both instruments by microscopic evaluation and evaluated a workflow for the routine urine analysis using the combination of UF-100 and Clinitek Atlas.

We analyzed 288 mainly pathological urine specimens by both test strip analyzer (Clinitek Atlas; Bayer Diagnostics) and UF-100 (Sysmex). The samples were examined within 3 h of arriving at the laboratory; no preservatives were used. We additionally reviewed 261 by microscopy.

The ranking system was used to compare the results from UF-100 and Clinitek Atlas (Table 1A). This ranking system was devised because cells were counted very precisely by UF-100 and, therefore, a differentiated classification was required to compare the test strips results.

The workflow strategy was to use both methods together and to detect implausible results by rank value differences between the Clinitek Atlas and UF-100 for erythrocytes and leukocytes separately. Differences in rank values of two or more were regarded as medically relevant. Specimens exhibiting this difference were reviewed by microscopy.

Discordant cases with lower rank values for strip results and higher rank values for flow cytometry were reviewed by microscopy. Microscopy was also performed in cases with higher ranks for strip results if large numbers of cells were seen. If cells were not seen in the UF-100, fresh samples were requested and examined. In some cases, the presence of lysed cells was assumed or excluded in concordance with the disease. Microscopic review was also performed when warning messages indicated that manufacturer specifications were being exceeded. The microscopy counts were performed after centrifugation (500*g* for 5 min; Madaus System). The cells were stained with Sternheimer supravital solution (Alcian Blue and Pyronin B; Oy Reagena) *(6, 7)*.

Our workflow strategy and rank classifications were then tested prospectively on 635 unselected routine specimens obtained over a 5-day period.

The dysmorphism and isomorphism criteria were examined on the UF-100 in 120 patients with confirmed renal or postrenal hematuria. The diagnostic sensitivity of these criteria for evaluating the localization of the hematuria was determined against phase contrast microscopy and established clinical diagnoses *(8–10)*.

Statistical calculations were carried out using SPSS for Windows *(11)* and the frequencies of binomial distribution *(12)*.

Red blood cell (RBC) and white blood cell counts were compared using both methods in 288 urine samples with a high frequency of pathologic results. In most cases (263 of 288 samples), there was agreement between the two methods based on our ranking system. The valid results were within the limits of 1.5 rank difference (see Table 1B). The 25 discordant cases are presented in Table 1B. The traditional workflow would have required microscopic review in 67% of the samples. Using the new combined approach, we reduced the microscopic review rate significantly, to 9% ($P < 0.05$).

This approach was further validated using 635 random samples. In this case, the microscopic review rate was 36% (n = 230). The use of both instruments reduced the review rate to 6% (n = 39; $P < 0.05$). Microscopy also clarified the situation in 25 specimens with discordant results (Table 1B).

Of the 263 concordant cases between the test strip and the UF-100, 248 were checked microscopically. Differing results for 6 samples for erythrocytes and 6 samples for leukocytes were obtained from 11 urine samples. In all cases, microscopic review revealed more cells than did the instruments.

The review algorithm of the UF-100 suggested microscopy in 142 samples (133 manufacturer-defined flags and

[1] Dedicated to Prof. Dr. E. Köttgen on the occasion of his 60th birthday.

Table 1. Ranking system and workflow criteria for use of the UF-100 and the Clinitek Atlas.

A. Ranking system for comparison of the results obtained with the UF-100 and the Clinitek Atlas

Rank	No. of cells according to test strip	No. of cells according to UF-100
−0.5	Class not defined	<4
0	Negative	4.1–20
0.5	~15	20.1–30
1	~70	30.1–70
2	~125	70.1–150
3	~500	150.1–900
4	Class not defined	>900

B. Criteria of workflow using the UF-100 and Clinitek Atlas in combination

	Study involving high frequency of pathologic results[a]	Validation study under routine conditions
Number of samples	288	635
Number of samples needing microscopy in traditional workflow because of pathologic results	192 (67%)	230 (36%)
Number of samples needing microscopy in workflow using both instruments because of discrepant results (≥2 ranks)	25 (9%)	39 (6.1%)
RBC discrepancies, total	13	21
Strips false positive	0	0
Strips false negative	0	4
UF-100 false positive	3	11
UF-100 false negative	10	6
WBC discrepancies, total	18	22
Strips false positive	0	0
Strips false negative	4	6
UF-100 false positive	4	11
UF-100 false negative	10	5
Device-related reasons for microscopic checks recommended by the UF-100 findings		142 samples
Manufacturer-defined flags		133
High total count, >40 000/μL		66
Nonlysed RBC, 20% or less		48
Abnormal conductivity		19
User-defined flags		103
SRCs		68
Microscopically confirmed		21 of 42
Pathologic casts		35
Microscopically confirmed		1 of 35
Necessary microscopic reviews of manufacturer- or user-defined flags		11

[a] For elaboration of roles for workflow.

103 user-defined flags). In most cases, user-defined flags were caused by increased numbers of SRCs and "pathologic casts" (Table 1B). In some of the specimens showing a review message for pathological casts, we found urate crystal aggregates. When compared with microscopic reviews, the UF-100 always flagged the presence of pathological casts, SRCs, or yeast cells. Most of the manufacturer-defined review flags (n = 66) were attributable to particle counts exceeding the upper limit of the UF-100 measurement range (>40 000/μL). These samples required reanalysis following dilution.

Other warning messages, e.g., "nonlysed RBC 20%" appeared in 48 of 635 samples. This message indicates strong lysis of RBCs, and the RBC value is extrapolated from exact fluorometric measurement of nonlysed RBCs (20%) and RBC fragments (ghosts, 80%). In some other cases, however, bacteria may be misclassified as RBC fragments, and the number of RBCs can be spuriously extrapolated by the UF-100.

The warning message "Abnormal DC Sensitivity" occurred almost exclusively in urine samples from newborns (7 of 14 samples) when the conductivity of the sample was abnormally low or high. Because our working algorithm assumed examination by both test strip and the UF-100, this warning flag could be ignored when test strip and UF-100 results were in concordance. This reduced the

necessary microscopic checks of flagged samples from 142 to 11 samples.

In this study, the localization of hematuria was based on the clinical diagnosis and morphological observations. These classifications were compared to the morphology flags obtained in the UF-100. The UF-100 has fixed algorithms for morphology flagging. In our study, the UF-100 correctly classified 37 of 53 postrenal (70%) and 66 of 86 renal hematurias (77%). In 20 cases, the UF-100 could not correctly classify hematuria because of low RBC counts.

These findings indicate that the combination of test strips with UF-100 flow cytometry can reduce and practically eliminate false-negative or -positive results that are obtained (~2%); these samples can be reviewed microscopically. The major reason for discrepant results was misclassification of particles (yeast or bacteria for lysed RBC). Scrutiny of the scattergrams, however, is crucial for correct classification of these particles. In addition to improved accuracy, our strategy yields quantitative results for particular elements in urine. This may be an improvement to the microscopic counts currently used for monitoring of renal diseases.

Because microscopic review of urine specimens requires 6–8 min per specimen, our approach reduces manual labor, and the majority of specimens can be analyzed using automated techniques. Furthermore, the reliability is improved by automation. The UF-100 displays several flags for microscopic review. In most cases, these reviews can be circumvented by using test strips in parallel and diluting samples with high total cell counts. However, the manufacturer-defined review flags for SRCs and pathological casts can be confirmed accurately only by microscopy. In our opinion, these findings are of less diagnostic importance because quantitative urinary protein determination offers higher diagnostic reliability in renal disease.

Renal and postrenal hematuria can be distinguished relatively well based on the isomorphism or dysmorphism flags of the UF-100. However, delay in analysis because of transportation problems combined with low RBC counts may also be a critical factor.

In summary, the combined sequential analysis of urine sample with a test strip analyzer and the UF-100 flow cytometer appears to be better than the standard procedures used to date. The main advantages are that the majority of specimens can be analyzed automatically, thus reducing manual labor and turnaround times. In specific cases, however, special microscopic techniques such as sediment analysis with or without supravital staining can still be used as auxiliary techniques.

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Rapid and Sensitive Immunoassay for the Measurement of Serum S100B Using Isoform-specific Monoclonal Antibody, *Miyoko Takahashi,* Andrea Chamczuk, Yuwen Hong, and George Jackowski* (Skye PharmaTech, Inc., 6354 Viscount Rd., Mississauga, ON L4V 1H3, Canada; * author for correspondence: fax 905-677-1674, e-mail empty@ICA.net)

S100 is an acidic calcium-binding protein with a molecular weight of 21 000, originally discovered by Moore *(1)* in the bovine brain. Today, >14 different S100 members are known *(2)*, of which S100A1 and S100B are the most studied *(3–6)*. Because a high concentration of S100B is present in the brain, S100 has been studied for use as a biochemical marker for central nervous system pathology. Numerous studies in the literature have suggested the clinical usefulness of measuring this protein in cerebrospinal fluid *(7–11)*, and in recent years, serum S100B has been reported as a useful marker for early detection of metastases of melanoma and cerebral complications from head injury, cardiac surgery, and acute stroke *(12–21)*.

With the advent of therapeutic treatments for stroke that either dissolve the clot or protect the brain, early diagnosis of stroke and the identification of appropriate patients for intervention are increasingly important. Existing assays lack sensitivity and ease of use and usually require ≥3 h to perform. We have, therefore, developed an ELISA that is rapid, highly sensitive, and S100B specific. This ELISA was used to retrospectively assess S100B concentrations in the serum of stroke patients.

Human S100B cDNA (Genome System), amplified by PCR, was cloned into vector pET28a (Novagen) and expressed in *Escherichia coli* BL-21 (DE3)pLysS (Novagen). Recombinant human S100B (rS100B) was isolated from host cells that were induced with 1 mmol/L isopropyl-thio-β-galactoside for 2 h at 37 °C and were lysed with a native buffer system. S100B was purified with an Ni-NTA affinity column.

The ELISA buffers were as follows: plate-coating buffer (100 mmol/L carbonate buffer, pH 9.6); phosphate-buffered saline (PBS; 1.5 mmol/L KH_2PO_4, 8.5 mmol/L $Na_2HPO_4 \cdot 2\ H_2O$, 2.7 mmol/L KCl, 137 mmol/L NaCl, pH 7.4); washing buffer (PBS containing 0.5 g/L Tween