Diagnosis of Acute Urinary Tract Infections Using Sysmex UF-100

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The authors report 29 months experience in the diagnosis of acute urinary tract infection using the UF-100 flow cytometer. Some 39,987 urine samples were examined. Each sample was analysed by UF-100 to assess bacteriuria and leukocyturia quantitatively. Samples with less than 25 leukocytes/μL and/or 3,000 bacteria/μL were considered negative and reported without further examination. Urine samples with leukocytes and/or bacteria over these cut-off values were submitted for culture. False negatives were detected by culture of resubmitted samples.

Of the total 39,987 samples examined, 28,471 (71.2%) were negative and 11,516 (28.8%) were positive using UF-100 for screening. Significant bacterial growth on culture did not occur in 1,563 (3.9%) of positive samples by UF-100 (false positives). There were 493 false negative samples (1.2%). The analytical performance of our protocol is described by the following results: sensitivity 0.95, specificity 0.95, positive predictive value 0.86, negative predictive value 0.98 and incidence of correctly classified samples 0.95.

In our opinion, quantification of leukocytes and bacteria in urine using the UF-100 flow cytometer is a useful method of screening for urinary tract infection.

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INTRODUCTION

Acute urinary tract infection (UTI) is common. Quantitative urine culture with identification of the etiological microbial agent and assessment of sensitivity to antibacterial drugs remain the standard laboratory procedures for diagnosis. Examination of urine samples is the most common microbiology test request in Clinical Pathology laboratory practice; moreover, usually around 70% of these urine cultures are negative[1].

In a previous report we have described the preliminary evaluation of a fully automated urine cell analyzer, UF-100 (Sysmex Corporation, Kobe, Japan) for the diagnosis of UTI[2]. In that study we suggested a diagnostic flow chart for the diagnosis of UTI. Briefly, we performed a UF-100-based screening for bacteriuria and leukocyturia; samples positive by the screening test were submitted to classical urine culture; samples negative by the screening test were not examined further and a negative result was sent to the physician within four hours of sample submission (***Fig. 1***).

Before the full adoption of this diagnostic protocol, we implemented a system for the identification of false negative results. If a patient specimen which was negative by UF-100 was resubmitted to our laboratories within 48 hours, this second sample was submitted to classical culture and if significant bacterial growth was observed, we considered this a false negative result. Following this modification the diagnostic protocol was adopted. This report describes our 29 months experience in the diagnosis of UTI after the adoption of this protocol.

MATERIALS AND METHODS

Study Design and Location

From October 2001 to February 2004 we examined 39,987 urine samples submitted to our Clinical Pathology laboratory for microbiological examination. Of these samples 17,994 (45%) were obtained from male patients aged between 3 months and 88 years (mean 56 years); 21,993 (55%) were obtained from female patients aged between 1 month and 92 years (mean 52 years). Of these samples, 16,794 (42%) were obtained from inpatients and 23,193 (58%) from outpatients. The great majority of these samples (38,761 - 96.9%) were voided urine specimens collected using the midstream technique; 479 samples (1.2%) were collected through a bladder catheter and 747 (1.9%) were collected using a paediatric device. The samples were collected in sterile containers and a 12 mL

aliquot was transferred into test tubes and analysed within one hour after sample submission.

Screening Methods

Each sample accepted for microbiological urinalysis was submitted to a screening procedure using UF-100. UF-100 is an automated analyser that performs urinalysis by flow cytometry. UF-100 aspirates 0.8 mL of urine and performs the analysis of cells (erythrocyte, leukocytes and epithelial cells), bacteria, casts etc., by electrical impedance for volume, forward light scatter for size and fluorescent dyes for nuclear and cytoplasmatic characteristics. The formed elements are categorised in two-dimensional space (scattergrams) on the basis of their size, shape, volume and staining characteristics[3-5]. A result was considered positive when bacteriuria and/or leukocyturia exceeded cut-off values of 25 leukocytes/μL and 3,000 bacteria/μL[1, 2, 6-10].

Microbiological Examination

Each sample submitted for microbiological culture was inoculated onto agar plates using 0.001 mL calibrated loops within 4 hours of collection. Both selective (McConkey agar [McC] and colistin-nalidixic acid blood agar [CNA]), and non-selective (CLED agar, cysteine, lactose, electrolyte deficient agar) media were used. After 24 hours at 37°C, cultures were quantified in CLED plates by evaluation of Colony Forming Units (CFU), as follows: from 0-10 colonies (under 10,000 CFU/mL, negative); from 11 to 100 colonies (from 10,000 to 100,000 CFU/mL, "moderate number"); over 100 colonies (over 100,000 CFU/mL, "large number") [11]. In cultures with a significant bacteriuria, a biochemical identification and an investigation into the sensitivity to antimicrobial drugs was performed using an automated system, the DADE Microscan (Dade International Inc., Sacramento CA, USA) [12].

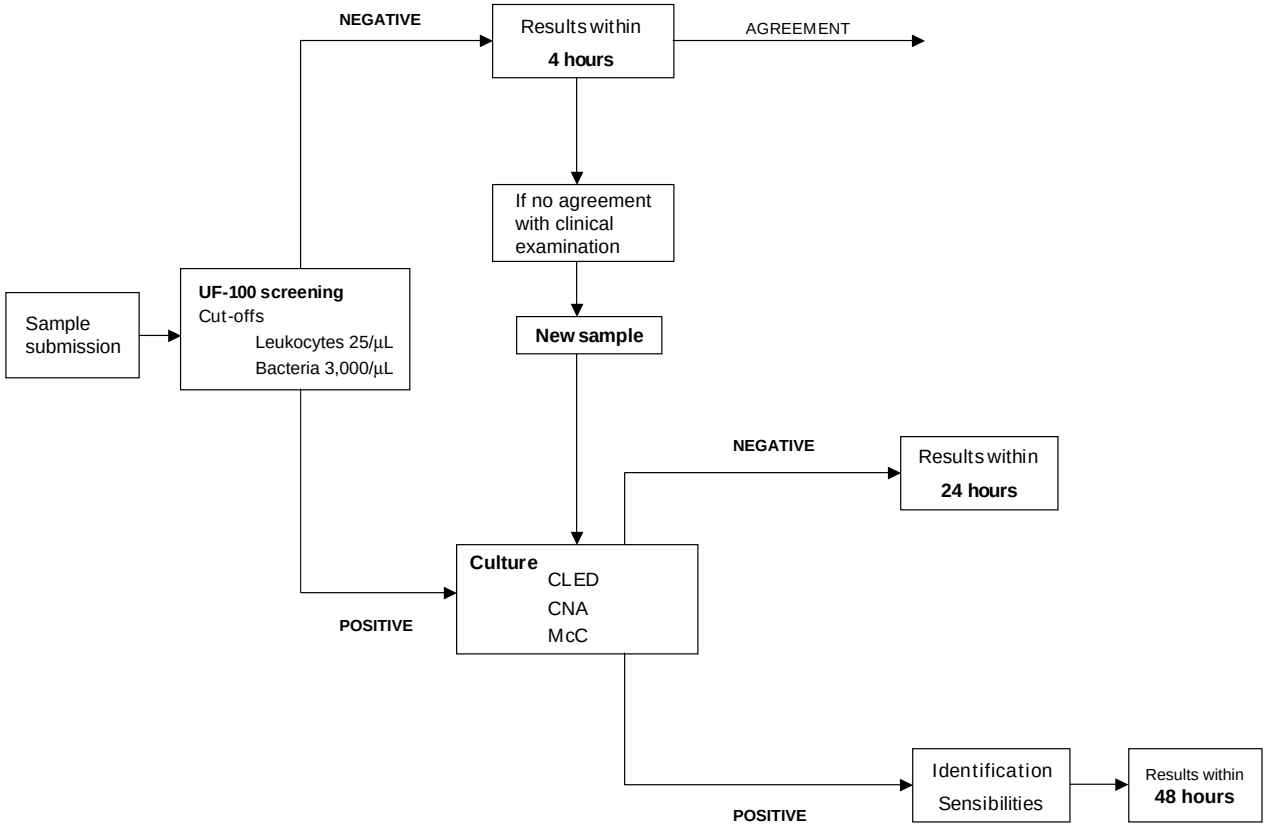

Fig. 1 *Description of the diagnostic protocol*

If the quantitative count of leukocytes and bacteria in a sample is under 25/μL and 3,000/μL, respectively, the sample is considered negative and the result is available to the physician within 4 hours after sample submission. If the negative result does not agree with the clinical picture, it is easy for the physician to resubmit a new sample for further examination before starting any treatment. All these repeated samples are submitted to a complete microbiological examination and if a positive result is obtained, a false negative initial screening result is considered. If the sample shows a leukocyte or bacteria count over 25/μL and 3,000 μL, respectively, the sample is considered positive and a complete microbiological examination is performed. If a significant bacterial growth is not observed, we consider this as a false positive screening test and the results become available to the physician within 24 hours after sample submission. If a significant bacterial growth is observed a biochemical identification of the bacterial strain and an assessment of the sensitivity to antimicrobial drugs is performed; these results then become available to the physician within 48 hours after sample submission.

Statistical Analysis

The analytical performance of our screening protocol was assayed by determination of sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and incidence of correctly classified samples (ICC). For comparison of proportions we used the Pearson's Chi Square test [13].

RESULTS

Of the 39,987 samples examined by UF-100, 28,471 (71.2%) were negative and these results were transmitted to the requesting physicians within four hours of specimen submission. Some 11,516 samples (28.8%) were positive by UF-100 and all were submitted to routine microbiological culture as described above. After cultivation, 1,563 (3.9%) of these samples did not produce significant bacterial growth and were considered to be false positives. These results were reported to the requesting physicians within 24 hours of sample submission. In 467 of these patients UF-100 showed only leukocyturia, in 397 bacteriuria and leukocyturia and in 699 only bacteriuria. In 9,953 (24.9%) samples significant bacterial growth (true positives) was observed. In these samples, identification of the bacterial strain and tests for susceptibility to anti-microbial drugs were performed; these results were reported to the requesting physicians usually within 48 hours after sample submission. The main bacterial strains observed in our studies are shown in ***Table 1***.

As described above, when a physician resubmits a specimen within 48 hours following a negative result by UF-100 screening, this second sample is cultured and if significant bacterial growth is observed, this is classified as a false negative result. Adopting these criteria, 493 false negative samples, (1.2%) were observed. The results obtained in these samples are shown in ***Table 1***. In 268 (54.4%) of those samples, bacterial growth from 10,000 to 100,000 CFU/mL was observed.

After statistical evaluation of the analytical performance of our protocol, sensitivity (SE) was 0.95, specificity (SP) was 0.95, the positive predictive value (PPV) was 0.86, the negative predictive value (NPV) was 0.98 and the incidence of correctly classified samples (CCI) was 0.95.

Table 1 *Bacterial strains observed in samples submitted for microbiological examination of urine*
The table shows separately the bacterial strains observed in 9,953 samples positive by the UF-100 screening test and in 493 samples negative by the UF-100 screening test but resubmitted by the physician on clinical grounds within 24 hours of a negative result.

	UF-100 Positives		UF-100 Negatives	
	Number	%	Number	%
Gram positives				
Enterococcus spp*	1495	15.0	136	27.6
Staphylococcus spp	191	1.9	18	3.7
Streptococcus spp	162	1.6	16	3.2
Other Gram positives	14	0.1	8	1.6
Gram negatives				
Escherichia coli*	5341	53.7	101	20.5
Klebsiella spp	789	7.9	24	4.9
Proteus spp	563	5.7	32	6.5
Pseudomonas spp*	499	5.0	86	17.4
Enterobacter spp*	376	3.8	31	6.3
Providencia spp	56	0.6	4	0.8
Morganella spp	61	0.6	3	0.6
Serratia spp	151	1.5	3	0.6
Citrobacter spp	82	0.8	3	0.6
Acinetobacter spp*	74	0.7	11	2.2
Salmonella spp	18	0.2	2	0.4
Other Gram negatives	56	0.6	4	0.8
Fungi				
Candida spp	25	0.3	11	2.2

* Difference statistically significant ($p<0.05$)

A comparison between the bacterial strains observed in samples positive and those negative by UF-100 screening shows that in the negative samples there is a higher prevalence of Gram-positive (mainly Enterococcus) and a lower prevalence of Gram-negative bacteria with a great drop in the percentage of Escherichia coli positive samples and an increased prevalence of other bacteria such as Pseudomonas spp and Acinetobacter spp.

DISCUSSION

Usually in clinical laboratory practice a great number of samples is submitted for urinalysis and microbiological examination, the great majority of which are not compatible with a diagnosis UTI. In this study we describe our experience in the diagnosis of urinary tract infection using, as a screening test, quantitative evaluation of bacteriuria and leukocyturia by the Sysmex UF-100 flow cytometer incorporated in the diagnostic protocol illustrated in ***Fig 1*** [2].

In our experience, 71.2% of specimens were negative by UF-100 screening and these results were available for the physician within four hours of sample submission. Therefore, in more than 2/3 of patients, UTI can be excluded and other diagnostic alternatives considered. This prevalence of negative samples accords well with other reports in the literature[1-2, 14-15]. Among these samples, there were 493 (1.2%) false negative results using the criteria described above. For these patients, particularly, it was possible for the requesting physician to identify a conflict between the initial laboratory result and the clinical picture and to submit a second specimen for microbiological culture. Obviously, the submission of these critical samples must be discussed with the Clinical Pathologist. We considered a sample to be false negative by UF-100 screening only if a significant bacterial growth was observed in the second sample. The results of microbiological examination of these screening-negative samples were considered separately from those obtained from screening-positive samples. Between these two groups of samples, we observed some statistically significant differences in the distribution of bacterial strains i.e. higher prevalence of Gram-positive bacteria (Enterococcus faecalis) and, among Gram-negative bacteria, a lower prevalence of Escherichia coli and a higher prevalence of Pseudomonas spp and Acinetobacter spp (see ***Table 1***). Furthermore, among the 493 false-negative samples, 268 (54.4%) revealed a low bacterial load (between 10,000 and 100,000 CFU/mL).

On UF-100 screening, 11,516 samples (28.8%) were positive. All were submitted to routine microbiological examination. After cultivation, 1,563 (3.9%) samples did not produce significant bacterial growth (false positives) and these results were available to the physicians 24 hours after sample submission. In 467 of these patients (females 63%) UF-100 screening showed only leukocyturia, in 397 bacteriuria and leukocyturia and in 699 only bacteriuria. In patients with pyuria without bacteriuria it is possible that leukocytes were coming from the genital mucosae rather than from the urinary tract, a consideration supported by the observation that the great majority of these samples was obtained from female patients. In patients with bacteriuria without pyuria, polymicrobial flora (more than three microbial strains) was usually observed suggesting contamination of the urine due to inappropriate collection, handling or storage. For the recognition of some of these false positive specimens, evaluation of the UF-100 leukocyte forward light scatter (Fsc) may be of great interest. Usually "fresh" leukocytes show a high Fsc and "old" leukocytes show a low Fsc. This difference appears to be due to cell volume changes. This observation suggests the presence of aged or dead leukocytes *"in vitro"* in urine stored for a long period or "*in vivo*" during prolonged stay of leukocytes in the bladder[16, 17].

Significant bacterial growth was observed in 9,953 [24.8%] samples (true positives). In these samples, identification of the bacterial strain and tests for susceptibility to anti-microbial drugs were performed as described above; these results were available to the physicians usually within 48 hours after sample submission. The distribution of the bacterial strains observed in our experience is classical with a large prevalence of Gram-negative bacteria; the main Gram-negative species was Escherichia coli and the main Gram-positive species was Enterococcus faecalis.

In our studies, UF-100-based screening showed an SE of 0.95, an SP of 0.95, a PPV of 0.86, an NPV of 0.98, and a CCI of 0.95. Of great importance, in our opinion, are the results obtained for NPV. A patient without significant leukocyturia and bacteriuria on UF-100 screening has a 98% probability to be free from UTI but may still be suffering from some other disorder. These data are in good accordance with our previous observations[2] and reports in the literature[14, 15, 18] although there are some exceptions[1]. In our studies, the results of UF-100-based screening are comparable to data obtained from culture examination.

The classic culture method requires 24 hours to produce a negative result whereas UF-100-based screening provides a negative result in a few minutes. Each year in our Clinical Pathology laboratory we perform approximately 90,000 urinalyses (chemical, physical and microscopy) and in about 20% there is a request for microbiological examination. Dipstick analysis of urine is performed using URIFLET strips and two Super Aution automated reflectance photometers (Menarini, FI, Italy). After this step, each sample is submitted to examination by UF-100. In this phase we count leukocytes and bacteria. In our experience, UF-100-based screening is inexpensive. Moreover, the quantification of bacteriuria and leukocyturia as a screening test for UTI does not call for a new and expensive procedure to be introduced into clinical laboratory practice, involving extra time and labour, but is a by-product of the routine examination of urine by flow cytometry.

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