ORIGINAL ARTICLE

Field evaluation of a second-generation cytometer UF-100 in diagnosis of acute urinary tract infections in adult patients

F. Manoni, S. Valverde, F. Antico, M. M. Salvadego, A. Giacomini and G. Gessoni

Clinical Pathology Department, ASL 14, Clinical Pathology Laboratory, Civil Hospital, Via Madonna Marina 500, 30019 Chioggia (Venice), Italy

Aims The authors evaluated the analytical performance of the Sysmex UF-100 cytometer vs. the diagnosis of urinary tract infections (UTI).

Methods We considered 2010 subjects, aged between 18 and 78, 870 males and 1140 females. The majority (90.2%) of the samples were voided urine specimens collected by using the midstream technique. Each sample was subjected to microbiological evaluation (culture + residual antibacterial activity), dipstick tests, UF-100 examination and microscopic observation. In order to obtain a final diagnosis of UTI these laboratory results were taken into consideration together with clinical data and patients' characteristics. The analytical performance of the laboratory tests was obtained by adopting this diagnosis as standard practice.

Results Out of the total 2010 subjects considered a clinical diagnosis of UTI was obtained in 529 cases (26.32%). The UF-100-based screening had sensitivity, 0.94; specificity, 0.93; positive predictive value, 0.83; negative predictive value, 0.98; and correctly classified incidence, 0.93.

Conclusions In our experience the results of the UF-100-based screening show a very good correlation with the diagnosis of acute UTI in adults patients.

Keywords Urinary tract infections, screening, UF-100, cytometer, pyuria, bacteriuria

INTRODUCTION

The main clinical manifestations of urinary tract infections (UTI) are dysuria, fever, suprapubic heaviness and pain; a microbiological examination of a urine sample is usually necessary to establish the aetiology of the disease [1]. For laboratory diagnosis of UTI it is of great importance to establish a definition of significant bacteriuria and pyuria. For bacteriuria, counts of more of 100 000 colony-forming units (CFU)/mL are usually considered significant [2,3]. For pyuria usually more than 5–10 white blood cells per high-power field (HPF) are considered significant [3].

In the clinical laboratory several screening tests have been devised for the rapid diagnosis of UTI, these tests can be classified into non-cultural methods and cultural methods [4]. The most common approach to a non-culture screening for UTI is the use of reagent dipsticks to detect the presence of nitrite and leucocyte esterase [5,6]. False negatives may result in the presence of infections by bacteria which do not produce nitrite, i.e. group D enterococci [7]. False positives may result in the presence of ascorbic acid, drug interference and overgrowth with nitrite-producing bacteria [8].

The aim of this study is to evaluate the feasibility of screening for the rapid diagnosis of UTI by using automated instrumentation for the study of the corpuscular parts of urinary samples. We evaluated a second-generation flow cytometer,

Corresponding author and reprint requests: G. Gessoni, Clinical Pathology Department ASL 14., Clinical Pathology Laboratory, Civil Hospital, Via Madonna Marina 500, 30019 Chioggia (Venice), Italy
Tel: +39 (0) 41 5534 406
Fax: +39 (0) 41 5534 401
E-mail: ctchioggia@libero.it

Sysmex UF-100 (Toa Medical Electronics, Kobe, Japan) vs. the diagnosis of UTI supported by laboratory data from urine culture, microscopic examination, routine chemical analysis, and clinical and demographic data such as age, sex, symptoms, urinary tracts abnormalities, and suspected diagnosis.

MATERIALS AND METHODS

Patient selection

We considered 2010 patients, aged between 18 and 78 years (mean 56.4 years), whose recently collected urine samples were submitted to our laboratory for diagnostic microbiological examination between January and August 1999. For each of these patients, their age, gender, symptoms, presence of underlying diseases (i.e. urinary tract anomalies or diabetes), therapy, etc. were recorded. The samples were obtained from outpatients (1130; 496 males and 634 females) and inpatients (880; 374 males and 506 females). The majority (1812; 90.2%) of samples were voided urine specimens collected using the midstream technique; 198 (9.8%) samples were collected through a bladder catheter. The samples were collected in sterile containers and a 12-mL aliquot was transferred into test tubes and analysed within 1 h.

Chemical and physical examination

Dipstick analysis of urine samples was carried out before flow cytometry analysis by using URIFLET strips and two Super Aution automated reflectance photometer (Menarini, Florence, Italy). The strips included reagent pads for semiquantitative assessment of relative density, pH, leucocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin and haemoglobin [9]. Each equipment could perform about 225 tests/h.

Microscopic examination

The classic microscopic examination was performed according to the NCCLS/95 guideline. After UF-100 analysis, each urine specimen (10 mL) was centrifuged at $400\,g$ for 10 min, and 9.5 mL of supernatant was discharged. In each specimen at least 20 random microscopic fields were examined at $\times 400$ (HPF) by the same experienced technician and the mean number of cells/HPF was calculated [10].

Culture of urine specimens

For microbiological examination, samples were inoculated on agar plates by using 0.001 mL calibrated loops within 4 h. Both selective [McConkey (McC) agar and colistin–nalidixic acid blood (CNA) agar] and non-selective cystine lactose electrolyte deficient agar (CLED) media were adopted. After 24 h at 37 °C cultures were quantified, in CLED plates, as follows: from 0 to 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') [11,12]. In cultures with a significant bacteriuria a biochemical identification and an investigation into the sensitivity to antimicrobial drugs was performed using an automated system: DADE Microscan (Dade International Inc. Sacramento CA USA) [13,14]. In each sample we studied the residual antibacterial activity (RAA) [15].

UF-100 examination

The Sysmex UF-100 is a second-generation automated analyser that performs an analysis of the formed elements in urine by using flow cytometry, this instrumentation is able to test 100 samples every hour. The UF-100 analyser sucks in 0.8 mL of uncentrifuged urine and performs the analysis [16]. The sample is transferred to the reaction unit and then is diluted four times with a special diluent to dissolve the crystalline contents. Then a mixture of two dyes, carbocyanine and phenanthridine, is added to the solution and the stained sample is entered into the flow cell where the urine's conductivity is measured. After this step the sample is entered into a flow cell to form a sheath flow which is created by passing a sheath liquid irradiated by an argon laser beam. The fluorescence, the pulse intensity and the pulse width of the forward-scattered light are measured [17]. These measures, together with the impedance data, are converted into three ranks of formed elements (Scattergram) [18]: erythrocytes, leucocytes, epithelial cells and bacteria are recognized from the scattergram of the forward-scattered light and the fluorescent light intensity. Epithelial cells are classified according to the forward-scattered pulse width and the fluorescence pulse width. For discrimination between casts and mucus it is important to consider the fluorescence pulse width. For erythrocytes, leucocytes, bacteria

and epithelial cells, the scattergrams and the counting result, expressed as a number of cells/mL, can be printed. Thereafter, the analyzer gives the operator further information about the morphology of red cells, the presence of sperm and/or pathological casts, etc. [19,20]. For the diagnosis of UTI using UF-100 we considered only two parameters among those previously described: quantification of bacteriuria and leucocyturia. In this study we considered that the cut-off value for UF-100 evaluation of pyuria is 25 cells/mL [21] and for bacteriuria, 100 000 cells/mL [22].

Statistical analysis

In this study the standard was the diagnosis of UTI obtained from integrating clinical considerations with laboratory results. In order to assess the analytical performance of UF-100 screening for bacteriuria and pyuria in the diagnosis of UTI we assessed the mean prevalence of this disease in the considered population (26.32%). Our statistical analysis included evaluation of specificity (SP), sensitivity (SE), positive predictive value (PPV), negative predictive value (NPV), and correct classification incidence (CCI). For comparison of means we adopted the Student's *t*-test; for comparison of proportions we adopted the Pearson χ^2 test; a value <0.05 is considered significant [23].

RESULTS

Of the 2010 patients considered, 529 (26.32%) were thought to be affected by a UTI. The prevalence of subjects affected by UTI was 33.52% among inpatients vs. 20.71% among outpatients; this difference was statistically significant ($P < 0.05$). Among the patients with UTI, 473 (89.41%) had bacteriuria over 100 000 CFU/mL and therefore, these patients matched the classic microbiological criteria for a diagnosis of UTI. In 56 (10.59%) patients bacteriuria was between 10 000 and 100 000 CFU/mL, but these subjects were considered as being affected by a UTI because of clinical considerations; 34 of these samples were positive for RAA and were still symptomatic despite the antimicrobial therapy. In these patients we observed a persistent infection sustained by yeast in eight, by fastidious Gram-positive organisms in 12 and by fastidious Gram-negative bacteria in 14. Among the 1491 subjects not considered affected by UTI, 1014 had bacteriuria under 10 000 CFU/mL, 432 between 10 000 and 100 000 CFU/mL and 35 had mixed bacteriuria with a microbial count over 100 000 CFU/mL. These 35 samples were considered not to be diagnostic for UTI because of contamination. In these samples we observed more than three bacterial strains, none of which accounted for almost 80% of the colonies [24]. These results are described in Table 1. By using the classic dipstick screening based on detection of

Table 1 Description of the final diagnosis of UTI in the considered population

Bacteriuria (CFU/mL)	Considerations	Number	%
Patients without urinary tract infections			
<10 000	Negative	1.014	50.45
10–100 000	Non-significant bacteriuria	432	21.49
>100 000	Mixed bacteriuria: contamination	35	1.74
Patients with urinary tract infections			
<100 000	Bacteriuria considered significant because of clinical considerations	56	2.78
>100 000	Significant bacteriuria	473	23.53

In the study population (2010 subjects) 529 patients (26.32%) were considered to be affected by a urinary tract infection.
Usually urine samples with bacteriuria under 10 000 CFU/mL are considered negative and bacteriuria under 100 000 CFU/mL is considered not significant.
In our study we considered 35 samples with bacteriuria over 100 000 CFU/mL not significant for UTI because of the observation of a mixed bacteriuria.
In our study we considered 56 samples with bacteriuria under 100 000 CFU/mL: significant for UTI because of clinical considerations.

Table 2 Analytical performance of various laboratory parameters with clinical diagnosis of UTI

	SE	SP	PPV	NPV	CCI
Dipstick screening: nitrite + esterase	0.64	0.88	0.63	0.89	0.82
Quantitative valuation of bacterial growth on CLED agar	0.89	0.98	0.93	0.96	0.95
Leucocyturia + Bacteriuria UF-100	0.94	0.93	0.83	0.98	0.93

We considered the analytical performance of the dipstick screening (evaluation of nitrite reduction and leucocyte esterase), the simple quantitative evaluation of bacterial growth on CLED agar, and the quantitative evaluation of bacteriuria and pyuria obtained from UF-100 analyser.

SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; CCI, correct classified incidence.

nitrites and leucocyte esterase in the urine sample we observed 171 (8.51%) false negatives and 184 (9.15%) false positive results. For this screening SE was 0.64, SP was 0.88, PPV was 0.63, NPV was 0.89 and CCI was 0.82.

The combination of the quantitative determination of bacteriuria and pyuria by UF-100 showed 29 (1.44%) false-negative results and 102 (5.07%) false-positive results. This combined screening had an SE of 0.94, an SP of 0.93, a PPV of 0.83, an NPV of 0.98 and a CCI of 0.93, with a clinical diagnosis of UTI. These results are shown in Table 2.

DISCUSSION

Usually in clinical laboratory practice a great number of samples are submitted for urinalysis and microbiological examination; around 70% of these samples are not compatible with a diagnosis of UTI. In this study the diagnosis of UTI obtained from clinical considerations and laboratory results was compared with the analytical performances of the simple evaluation of bacterial growth on CLED agar, the dipstick screening for nitrite and esterase, and the quantitative evaluation of bacteriuria and pyuria obtained by using UF-100. By using the simple evaluation of the number of CFU/mL observed on CLED agar we observed 35 (1.74%) false-positive results and 56 (2.79%) false-negative results. In our experience this test had an SE of 0.89, an SP of 0.98, a PPV of 0.93, an NPV of 0.96 and a CCI of 0.95. The analytical performance of dipstick screening is relatively satisfactory. There were 171 (8.51%) false-negative results and 184 (9.15%) false-positive results. In our experience the dipstick screening SE was 0.64, SP was 0.88, PPV was 0.63, NPV was 0.89 and CCI was 0.82; these results appeared to be in good accordance with data from the literature [5–7,11–13]. Quanti-

tative determination of bacteriuria and pyuria by UF-100 showed 29 (1.44%) false-negative results and 102 (5.07%) false-positive results. This screening had an SE of 0.94, an SP of 0.93, a PPV of 0.83, an NPV of 0.98 and a CCI of 0.93. Our results show that the UF-100 screening has a better correlation with the diagnosis of UTI, not only in comparison with the dipstick screening test, but also in comparison with the simple evaluation of bacterial growth on CLED agar. In our study this screening demonstrated an NPV of 0.98. So a subject without significant leucocyturia and bacteriuria in UF-100 screening has a 98% probability of being free from UTI.

These data are of great practical importance. Indeed they may be significant in establishing a routine to dismiss microbiological examination of urine specimens with a negative result in UF-100 screening [25–27]. In our experience the use of UF-100 as a screening test for UTI showed only 29 false-negative and 102 false-positive results. Among the 29 false-negative results, 20 were observed in patients with UTI but with low bacterial counts (<10 000 CFU/mL). In these 20 samples the evaluation of the bacterial growth (cut-off at 100 000 CFU/mL) on CLED agar gave a negative result too. We observed only nine false-negative results in samples with high bacterial counts (>100 000 CFU/mL). Among the 102 false-positive results, 35 were because of the presence of polymicrobial flora contaminating the urine samples, 59 were due to detection of pyuria (but not bacteriuria) without UTI; 51 of these subjects were female so it is possible that the leucocytes had come from the genital mucosae rather than from the urinary tract. In eight subjects UF-100 gave a false-positive result because of the detection of bacteriuria without pyuria and without UTI, probably because of multiplication of bacteria due to inappropriate handling or storage of the urine sample.
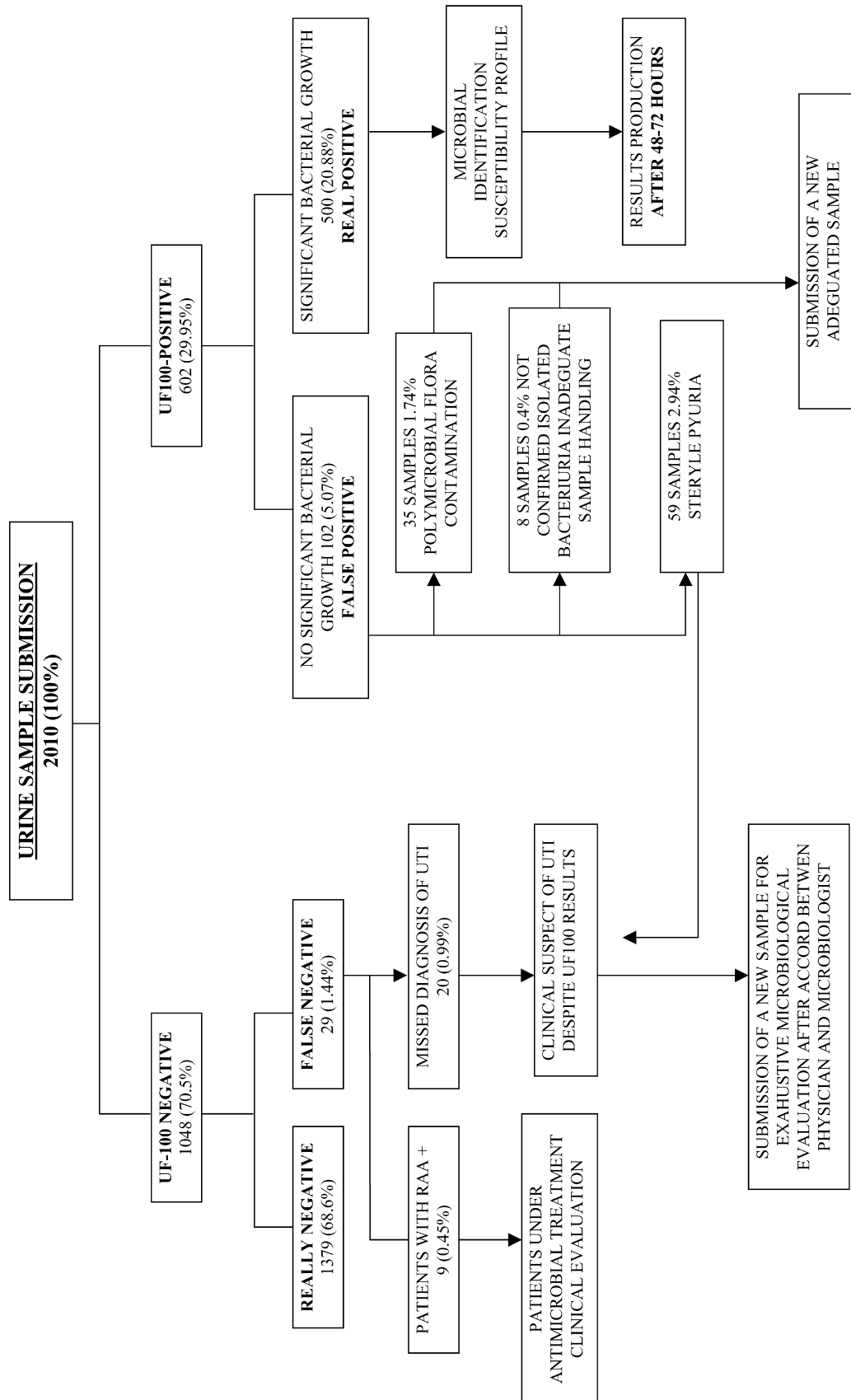
Figure 1 Flow chart based upon the authors' experience with urinalysis performed using the UF-100 instrumentation.

In Figure 1 a flow-chart for diagnosis of UTI based on the results of this study was suggested. We considered 2010 urine samples, out of which 1408 (70.05%) were negative using the UF-100 screening. For all these samples it was possible to produce results within a few minutes of the submission of the sample for a microbiological evaluation. Out of these samples 1379 were actually (68.61%) negative. Twenty-nine samples gave false-negative results (1.44%); out of these, nine (0.45%) came from patients receiving antibacterial treatment for a previous diagnosis of UTI (these patients gave a positive result for the RAA test in urine). The UF-100-based screening failed in only 20 samples (0.99%) for the diagnosis of UTI in untreated patients. For these subjects, the short time required for the laboratory results, allows the physician to critically assess the laboratory data and to decide on the submission of a new sample in order to obtain a complete, careful microbiological examination before starting any treatment. Obviously, the submission of these critical samples must be discussed with the clinical pathologist. On the other hand, 602 samples (29.95%) were positive using the UF-100 screening; all these samples were submitted to a culture examination; in 500 samples (20.88%) we observed a significant bacterial growth. These were true positive results. In all these samples a biochemical and serological identification of the bacterial strain was performed, together with an in vivo susceptibility test for antibacterial drugs; a complete result may be available to the physician within 48–72 h of the sample's submission. In 102 samples (5.07%) we did not observe significant bacterial growth: these were false-positive results. In 35 (1.74%) of these false-positive results we observed a polymicrobial flora, and so there was no need for further tests. Perhaps in these subjects the need for a correctly recovered new sample should be evaluated. In eight (0.4%) samples we observed the presence of bacteriuria without pyuria. The authors suggest that in these samples the incongruent result may be due to an incorrect handling of the sample used for the UF-100 screening. In 59 (51 female) samples (2.94%) we observed a 'sterile pyuria'; the absence of significant bacteriuria was confirmed by culture examination but the presence of leucocytes remained unexplained. It is possible that these white blood cells came from the mucosae of the female genital tract. These samples need further examination because it is possible

that an infection was sustained from fastidious micro-organisms such as *Neisseria gonoree*, *Chlamydia trachomatis*, *Microbacterium tuberculosis*.

In conclusion, in our experience the results of the UF-100-based screening are comparable to data obtained from culture examination based on the simple evaluation of bacterial growth on CLED agar (cut-off 100 000 CFU/mL). The classic culture method requires 24 h to produce a result, whereas the UF-100-based screening gives a result within a few minutes, with obvious benefit for patients and physicians. In our clinical laboratory the examination of the urine using the UF-100 system is economical because the cost of this test is only 0.69 Euro, inclusive of equipment rental and reagents. Moreover, the quantification of bacteriuria and pyuria used in this study as a screening test for UTI does not call for a new and expensive test to be introduced in the clinical laboratory, involving extra time, labour and costs, but it is a by-product of the examination of the corpuscular portion of urine samples when using a flow cytometer [28,29].

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