

# Improving the efficiency and efficacy of pre-analytical and analytical work-flow of urine cultures with urinary flow cytometry

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## SUMMARY

Quantitative urine culture is one of the most frequently requested tests in microbiology laboratories. An automated system for screening purposes is strongly needed to save technical staff time and obtain rapid results.

Our study investigated 1.047 urine samples collected from inpatients and outpatients with a commercial vacutainer system (Becton Dickinson, Milan, Italy) and compared a second-generation flow cytometry (Sysmex UF100, Dasit, Cornaredo, Italy) with standard urine culture tested on agar plated by means of 10 microliter loop. The specimens were screened and cultured on receipt.

The results obtained with Sysmex UF-100 are very interesting, especially if this analyzer is used as a screening method for negative urine samples, and comparable to data obtained from culture examination. In fact, considering together bacteria and leukocyte count ( $>4500$  bacteria and/or  $>50$  leukocytes/ $\mu\text{L}$ ) we obtained a negative predictive value of 99.5% in comparison with the standard culture method.

The classical culture method needs 24 hours for a result, whereas the Sysmex UF-100 analyzer gives results in a few minutes, thus reducing the microbiology turn around time (TAT) with obvious benefits for patients and physicians.

**KEY WORDS:** Urinary tract infections, Urinary flow cytometry, TAT

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## INTRODUCTION

Urinary tract infections (UTI) are the infectious diseases with the highest incidence in the hospital and community population (Kouri *et al.*, 2000; Camporese, 2002).

The aims of the microbiology laboratory in the management of UTI are to identify and quantify the aetiological agents of infection, to offer a susceptibility testing for antimicrobial treatment and to follow the course of the disease or the effects of the prescribed antibiotic treatment.

Although the incidence of UTI is high, a large proportion of the samples tested by a routine microbiology laboratory will show no evidence of infection with up of the 80% of the specimens with negative results for urine culture. Therefore a rapid and reliable screening method is useful to screen out negative samples, reducing unnecessary testing.

There is still no agreement in literature over which test should be considered the most suitable and rational for UTI screening, even though in our opinion the detection of bacteriuria together with a quantitative evaluation of pyuria by microscopic examination should be preferred (Kouri *et al.*, 2000; Camporese, 2002; Huicho *et al.*, 2002).

Today, however, new automated methods based on advanced technologies are available for UTI screening (Kouri *et al.*, 2000; Camporese, 2002; Huicho *et al.*, 2002) with technical and analytical

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specifications that could not even be imagined just a few years ago.

However, the introduction of a new automated analyzer in a microbiology laboratory for UTI screening purposes must be carefully evaluated not only in terms of analytic and diagnostic performance. Due to the higher cost per test of these systems with respect to traditional plate culture, a microbiology laboratory oriented towards automation should evaluate the impact of a new analyzer in a broader programme for the reorganization of pre-analytical and analytical workflow, including software connection to the LIS (*Laboratory Information System*) and to the hospital wards, to improve the quality and efficiency of diagnostic procedures reducing turnaround times and providing the same high quality of results without losing valuable time treating the patient (Bruschetta *et al.*, 2006; Camporese, 2004).

## MATERIALS AND METHODS

A total of 1.047 consecutive urine samples from inpatients and outpatients were tested in parallel, upon receipt with the Sysmex UF-100 urine flow cytometry analyzer and with the standard urine culture method.

### Urine specimens

Urine specimens were collected in a sterile collection cup with an integrated device (Becton Dickinson, Milan, Italy) that allows the automated transfer of urine to a vacutainer tube (Figure 1). For each specimen a single vacutainer tube without preservatives was used for automated urinalysis with the Sysmex UF-100 and for microbiology examination.

The reason we adopted this procedure is based on the results obtained in our previous evaluation (Bruschetta *et al.*, 2006) where we found, on 564 samples collected in double, a total agreement of culture results between specimens plated after being analyzed by the UF-100 and specimens directly plated. Furthermore, in our experience, the use of boric acid as preservative can affect the cut-off values used for the screening method, depending on the level of crystallisation of borates used by different manufacturers of urine collection and transport devices. This could lead to reassess the cut-off values whenever, for

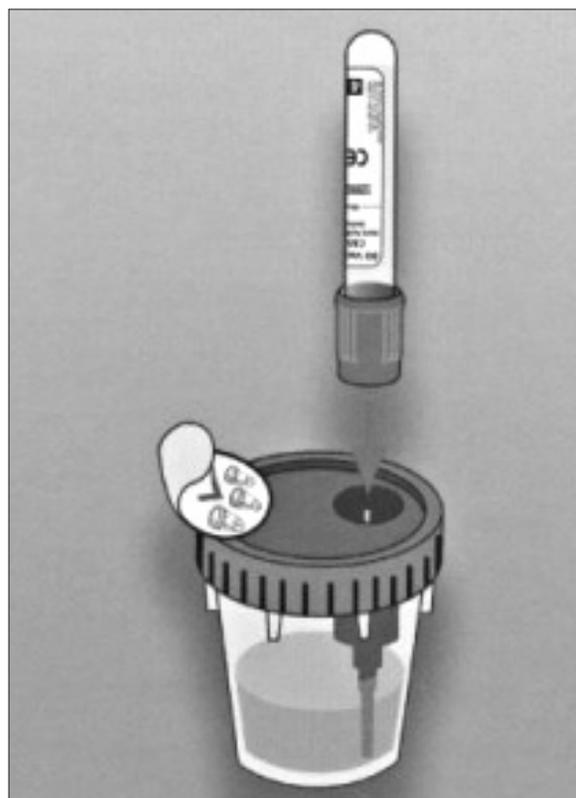


FIGURE 1 - Sterile urine collection cup with a screw cap to minimize the risk of leakage and contamination. The collection cup has a specific holder where a vacutainer tube can be connected for the automated transfer of urine specimen.

any reason, the need arises to use containers supplied by different manufacturers.

### Quantitative urine culture

For microbiological examination, urine specimens were inoculated with a 10 mL calibrated loop onto CLED agar, Columbia CNA +5% sheep blood plates and germ agar (Kima, Padua, Italy) for the antimicrobial residual activity evaluation. The plates were incubated for 24h at 37°C and examined for significant bacteriuria. Culture results were reported as positive if they contained  $\geq 10^5$  CFU/mL of one or two potentially pathogenic microorganisms.

### Automated urinalysis with the Sysmex UF-100

The Sysmex UF-100 is a fully automated analyzer that identified and count the following formed elements in urine by using flow cytometry and

impedance methods (Kouri *et al.*, 1999; Regeniter *et al.*, 2001; Manoni *et al.*, 2002) bacteria, leukocytes, yeasts, erythrocytes (RBCs), epithelial cells, casts, epithelial transitional cells, crystals and sperm cells.

Up to 100 samples/hour can be processed in automated mode requiring at least 4 mL of uncentrifuged native urine sample, or 1 mL in case of manual analysis.

Previous studies by other authors have shown the usefulness of Sysmex UF-100 in the diagnosis of UTI by the use of different cut-off values for the quantification of bacteriuria and leucocyturia (Kouri *et al.*, 1999; Regeniter *et al.*, 2001; Manoni *et al.*, 2002; Manoni *et al.*, 2001; Valverde *et al.*, 2003; Rossetti *et al.*, 2002).

In the present study, samples were defined as positive for UTI screening if the results were greater than the following cut-off values: 4500 bacteria/mL and/or 50 leukocytes/mL.

### Statistical analysis

The comparison of Sysmex UF-100 with bacterial culture results was evaluated in terms of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values. Diagnostic performances were evaluated in terms of sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV). Each value was expressed as a percentage.

## RESULTS

Of the 1047 urine samples evaluated, 247 (23.6%) were culture positive and 800 (76.4%) were culture negative. Overall, 448 (42.8%) urine samples were positive and 599 (57.2%) were negative with the Sysmex UF-100.

The comparison of Sysmex UF-100 and bacterial culture results (Table 1) demonstrated that:

- 244 samples (23.0%) were both culture and Sysmex UF-100 positive (True Positive, TP);
- 596 samples (56.2%) were both culture and Sysmex UF-100 negative (True Negative, TN);
- 204 samples (19.5%) were culture negative and Sysmex UF-100 positive (False Positive, FP);
- 3 samples (0.3%) were culture positive and Sysmex UF-100 negative (False Negative, FN);

By using bacterial culture results as the reference, diagnostic performances for Sysmex UF-100

TABLE 1 - Quantitative urine culture versus Sysmex UF-100 results.

Sysmex UF-100 results	Quantitative urine culture results	
	Positive	Negative
Positive	244 (TP)	204 (FP)
Negative	3 (FN)	596 (TN)

TP: true positive, FN: false negative, FP: false positive, TN: true negative.  
 Sensitivity =  $TP/(TP+FN) = 244/(244+3) = 98.8\%$   
 Specificity =  $TN/(TN+FP) = 596/(596+204) = 74.5\%$   
 Negative predictive value NPV =  $TN/(TN+FN) = 596/(596+3) = 99.5\%$   
 Positive predictive value PPV =  $TP/(TP+FP) = 244/(244+204) = 54.5\%$

were: sensitivity (SE) 98.8%, specificity (SP) 74.5%, negative predictive value (NPV) 99.5%, positive predictive value (PPV) 54.5%.

Therefore, as the aim of the study was to assess the diagnostic performance of Sysmex UF-100 in the identification of the true negative samples, because the positive ones are cultured in any case, the Sysmex UF-100 seems to provide an excellent screening method for negative samples.

Among the 204 false positive samples obtained by UF-100, in 155 the leukocytes count was  $>50/\mu\text{L}$ , in 144 the bacterial count was  $>4500/\mu\text{L}$ , in the range shown in Table 2. It is important to underline that 16% of false positive results were positive at the antimicrobial residual activity evaluation, and more of 20% showed at least one of over-normal range parameters such as RBCs or turbidity, or crystals (data not shown).

From the re-examination of the false positive results, we found that a fair proportion (15.6%) of the false positive samples had only a leukocytes count greater than  $50/\mu\text{L}$  (data not shown). We therefore decided to re-assess the diagnostic performances of the Sysmex UF-100 using a new cut-off value for leukocytes.

In fact, increasing the cut-off value to 100 leuko-

TABLE 2 - Leukocytes and bacterial count of false positive results generated by UF-100 analysis.

Leukocytes count ( $>50/\mu\text{L}$ )	50-1000	1000-50000
N° of samples	142	13
Bacterial count ( $>4500/\mu\text{L}$ )	4500-10000	10000-100000
N° of samples	92	52

cytes /mL we obtained a lower number of false positive samples (from 204 to 172, corresponding to 16.4% of the total number of samples), 7 reducing the percentage of samples that need to be cultured from 42.8% to 39.7% with a recovery rate of about 3%. As no true positive at all were lost using the new leukocytes cut-off value, the negative predictive value remained the same and the positive predictive value improved slightly, rising from 54.5% to 58.6%.

There were only 3 false negative cultures that produced 105 CFU/mL of *Streptococcus agalactiae*, all in pregnant women, thus the false negative data obtained were absolutely not relevant in terms of clinical impact.

## DISCUSSION

A considerable number of the specimens examined in a routine diagnostic microbiology laboratory are urine. The diagnosis of urinary tract infection is usually based on the quantitative culture of urine samples. In our microbiology laboratory, urine culture testing accounts for 19% of the daily workload for a total of 18.628 urine samples analyzed in the year 2007.

With the view to reorganizing the whole management process on urine microbiology examination, we evaluated several materials and methods including collection, transport, handling and sorting of specimens as well as a new automated analyzer for UTI screening.

The results of the present study, together with those obtained in our previous evaluation (Camporese *et al.*, 2004), allowed us to obtain a significant rationalization in the reorganization of the pre-analytical and analytical phases, significantly improving the efficiency and effectiveness of the whole analytical process on urine samples submitted for microbiological investigation.

After having evaluated a new system for the collection and transport of urine specimens, we chose a more advanced and practical sterile collection cup (Becton Dickinson, Milan, Italy) with an integrated transfer device that allows the automated transfer of urine to a single vacutainer tube (Figure 1) without boric acid as preservative. This system ensured a significant improvement in the handling of the sample by the patient and in the safety of sample handling and transport from

the hospital wards and from the several collecting sites of the local area.

The use of a single vacutainer tube for the immediate screening by the Sysmex UF-100 analyzer and, in case of positive results, for urine culture examination has also significantly improved the pre-analytical phase in our laboratory decreasing the number of samples that need to be accepted and sorted, the spaces required for sample transport and storage, with a fairly good economic saving on the whole analytical process.

From the analytical point of view, the results of this study confirm the usefulness of the Sysmex UF-100 analyzer as a screening method for UTI. The high negative predictive value (99.5%) and the low percentage of false negatives (less than 0.3% of the total samples analyzed), both absolutely fundamental to guarantee the diagnostic efficacy of the screening process, allow us to claim that the Sysmex UF-100 is able to reach the diagnostic excellence that we set out to obtain.

The percentage of false positives (equal to 16.4% of the total samples analyzed after the revision of the cut-off value to 100 leukocytes/mL) does not interfere, if only marginally, with the efficiency of the screening process.

From the management point of view, one of the most interesting features that we have experienced with the introduction of the Sysmex UF-100 in our laboratory is its major contribution to improving the global turn around time (TAT). The results from the analyzer are practically available in "real time", within a few minutes of the sample admission, and can be sent to the LIS for validation and subsequent immediate reporting in case of negative results.

Considering that the TAT of positive samples in our laboratory is already at an excellent level with respect to the latest international guidelines (Kouri *et al.*, 2000; Camporese, 2002; Camporese *et al.*, 2003; Camporese *et al.*, 2004), with the Sysmex UF-100 we can now guarantee a significant reduction of TAT for negative samples, as 57.2% of samples can be reported as negatives practically in real time.

Within our microbiology laboratory, considering the workload in 2007, this percentage is extremely high and represents a total of 10.655 samples. For physicians this should mean prompt reporting of normal samples and improvement in the quality of patient care.

In conclusion, urine flow cytometry can be considered a novel high-performance and high throughput screening procedure in microbiological laboratories allowing a significant reduction in costly and unnecessary tests. However it cannot replace urine culture, that remains the gold standard to confirm urinary tract infection.

For this reason we have requested that our regional health care institution include the reimbursement for this diagnostic test within the same regional code corresponding to the "detection of bacteria by microscopy examination with special staining procedures".

We believe this choice is totally in agreement with the purpose of urine flow cytometry in microbiology laboratories, since the quantification of bacteriuria and/or pyuria by microscopy examination, with or without staining of uncentrifuged urine, is traditionally considered the simplest and most effective screening method for the exclusion of urinary tract infection (Kouri *et al.*, 2000; Camporese, 2002; Huicho *et al.*, 2002).

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