Diagnosis of Bacteriuria and Leukocyturia by Automated Flow Cytometry Compared with Urine Culture

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Urinary tract infection (UTI) is a widespread disease, and thus, the most common samples tested in diagnostic microbiology laboratories are urine samples. The “gold standard” for diagnosis is still bacterial culture, but a large proportion of samples are negative. Unnecessary culture can be reduced by an effective screening test. We evaluated the performance of a new urine cytometer, the Sysmex UF-1000i (Dasit), on 703 urine samples submitted to our laboratory for culture. We compared bacteria and leukocyte (WBC) counts performed with the Sysmex UF-1000i to CFU-per-milliliter quantification on CPS agar to assess the best cutoff values. Different cutoff values of bacteria/ml and WBC/ml were compared to give the best discrimination. On the basis of the results obtained in this study, we suggest that when the Sysmex UF-1000i analyzer is used as a screening test for UTI the cutoff values should be 65 bacteria/ml and 100 WBC/ml. Diagnostic performance in terms of sensitivity (98.2%), specificity (62.1%), negative predictive value (98.7%), positive predictive value (53.7%), and diagnostic accuracy (73.3%) were satisfactory. Screening with the Sysmex UF-1000i is acceptable for routine use. In our laboratory, we have reduced the number of bacterial cultures by 43%, speeded up their reporting, and decreased the inappropriate use of antibiotics.

The urinary tract is the most common site of infection, and urine culture is the standard diagnostic test for urinary tract infection (UTI). The growing need to enhance the performance of urine culture combined with the need to free up resources by rejecting negative samples quickly and economically has drawn attention to solutions that can be used as screening tests to reduce the number of unnecessary culture tests. In this study, we sought to evaluate and optimize the performance of the Sysmex UF-1000i (Dasit) on 703 urine samples submitted at the same time to bacterial culture analysis.

MATERIALS AND METHODS

A comparison between the Sysmex UF-1000i urinary cytometer and urine culture was conducted on 703 samples, 128 of which (18.2%) were from hospitalized patients and 575 (81.8%) from outpatients. The urine samples were collected in sterile containers using the technique of midstream capture and were processed within 2 h of collection (5, 10). Each sample was divided into two sterile tubes with a vacuum system (Vacutainer; Becton-Dickinson); one tube was used for urine culture and the other for analysis by the Sysmex UF-1000i.

The urine culture was performed by sowing 10 μl of the urine sample on chromogenic agar plates (CPS agar; bioMérieux) with calibrated loops. CPS agar is a specific medium for isolation of bacteria in urinary samples. It allows identification of several bacteria—Enterobacteriaceae and Gram-positive bacteria—and it contains specific chromogenic substrates for detecting different enzymatic activities. The resulting colonies are well isolated and easily identifiable by differentiating colors (1). All plates were incubated for 24 h at 37°C, and the results were expressed as the number of CFU per milliliter.

According to the manufacturer’s recommendations and previous reports (2, 12), colonies were identified as follows: Escherichia coli, pink to burgundy color; Staphylococcus spp., small turquoise colonies; KESC group (Klebsiella spp., Enterobacter spp., Serratia spp., and Citrobacter spp.), large green colonies; Proteus, light-brown to dark-brown colonies; Streptococcus agalactiae, small violet colonies; Staphylococcus aureus, yellow colonies; Candida spp., white colonies. Colonies whose appearance did not conform to any of these descriptions were recorded as “other” (1).

The Sysmex UF-1000i system analyzes 0.8 to 1.2 ml of sample in batch by combining flow cytometry with fluorochrome (polymethine dyes) and impedance analysis. The results are available in 1 min for one sample. The application of these technologies allows the discrimination and quantification of bacteria, erythrocytes, leukocytes (WBC), epithelial cells, casts, crystals, fungi, and sperm in a urine sample (7, 8).

The data obtained with the Sysmex UF-1000i were compared with those obtained from seeding urine sample on CPS agar. Samples were considered positive if they contained ≥10^5 CFU of urinary pathogens/ml (9, 11, 13). Data for each patient were included in an Excel spreadsheet reporting patient details, leukocyturia and bacteriuria on the Sysmex UF-1000i, urine culture results, and the types of microorganisms isolated, including yeasts.

Statistical analysis of the data was performed with parametric and nonparametric methods, as appropriate. Cohen’s correlation coefficient K and receiver operating characteristic (ROC) curves for bacteriuria and leukocyturia on the Sysmex UF-1000i were also evaluated in order to establish their cutoffs and the diagnostic performance of the method in use by determining sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA). The evaluation of statistical data was carried out with the software Analyze-it (release 2.20) and SPSS (release 12.0).

RESULTS

In May 2009, 703 samples were analyzed, 128 (18.2%) belonging to hospitalized patients and 575 (81.8%) belonging to outpatients. Overall, samples from female subjects were more prevalent (496; 70.6%) than samples from males (207; 29.4%).

By analyzing the distribution of samples based on age, we observed different frequency peaks, in particular, at ages 0 to 5, 25 to 45, and 60 to 85 years. This trimodal distribution is exactly what one would expect with regard to urine cultures received in the laboratory in a multifaceted center.
In total, 486 (69.1%) samples were negative at urine culture (no growth or a bacterial count of \(<10^5\) CFU/ml) and 217 (30.9%) were positive, with bacterial counts of \(10^4\) to \(10^5\) CFU/ml. Among these, 26 samples (3.7%) had bacterial counts between \(10^4\) and \(10^5\) CFU/ml and 31 (4.4%) were considered contaminated because they showed 3 or more types of colonies without a dominant species and a bacterial count of \(10^5\) CFU/ml. According to the Scottish Guidelines (13), the samples for which it was not possible to attribute clinical significance to the pathogens isolated were deemed to have been due to patient misinterpretation of urine collection instructions.

The analysis of positive samples stratified by sex showed a frequency of positivity of 32.8% (163/496) in samples from female subjects and 26.1% (54/207) in samples from male subjects. The prevalence of positive urine culture (samples with positive results/total samples \(\times 100\)) was 30.8%. Table 1 shows a comparison between the quantification of bacteria on the Sysmex UF-1000i and urine culture with parametric and nonparametric analyses. Similarly, Table 2 shows a comparison between the quantification of WBC on the Sysmex UF-1000i and the results of urine culture with parametric and nonparametric analyses.

The urine culture results on CPS agar were classified into four groups based on the bacterial count (\(10^4\) to \(10^5\) CFU/ml, \(10^4\) to \(10^5\) CFU/ml, and contaminated), as shown in Tables 1 and 2 with the related Sysmex results. The statistical analysis of the four groups is reported as follows: parametric (mean, 95% confidence interval [CI], and standard deviation [SD]) and nonparametric elements (median, 95% CI, minimum and maximum values, and interquartile range [IQR]).

Figure 1 shows the distribution of values of bacteriuria (cells/\(\mu l\)) obtained on the Sysmex UF-1000i compared to the results of urine culture. Similarly Fig. 2 shows the distribution of values for leukocyturia (cells/\(\mu l\)) given by the Sysmex UF-1000i, reported on the vertical axis, compared to urine culture results, reported on the horizontal axis.

The numbers of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) and the values for SE, SP, PPV, NPV, and DA calculated at different values of bacteriuria and leukocyturia are summarized in Table 3. Sensitivity was calculated as TP/(TP + FN), specificity was calculated as TN/(TN + FP), PPV was calculated as TP/(TP + FP), NPV was calculated as TN/(TN + FN), and DA was calculated as (TP + TN)/(TP + TN + FP + FN). These parameters are reported as percentages.

Figure 3 shows the ROC curve for leukocyturia and bacteriuria on the Sysmex UF-1000i. The Sysmex UF-1000i has a better ability to discriminate bacteremia than leukocyturia, as

| Table 1. Comparison of bacteriuria obtained by the Sysmex UF-1000i against urine culture on chromogenic agar (CPS) |
|----------------|----------------|----------------|----------------|
| Statistic\(^a\) | Sysmex UF-1000i result against urine culture | \(<10^4\) CFU/ml | \(10^4\)–\(10^5\) CFU/ml | \(\geq10^5\) CFU/ml |
| | \((n = 486 samples)\) | \((n = 26 samples)\) | \((n = 160 samples)\) | \((n = 31 samples)\) |
| Parametric Mean | 541.9 | 418.9 | 15,016 | 5,753 |
| 95% CI | 359.8–724.1 | 216–625.3 | 12,319.7–17,712.3 | 2,412.8–9,093.2 |
| SD | 2,044.4 | 510.9 | 17,268.6 | 9,106.3 |
| Nonparametric Median | 25.9 | 274.3 | 7,532.2 | 2,121.5 |
| 95% CI | 20.9–33.9 | 121.9–459.7 | 4,312.4–11,212 | 421.6–3,356.3 |
| Minimum | 0.2 | 13.9 | 12.9 | 68.8 |
| Maximum | 25,789.8 | 2,275.3 | 86,851.7 | 36,622.7 |
| IQR | 195.9 | 349.2 | 21,556.9 | 6,718.9 |

\(^a\) For bacterial count by the Sysmex UF-1000i.

| Table 2. Results obtained by comparing the leukocyte counts of the Sysmex UF-1000i with quantitative urine culture on chromogenic agar (CPS) |
|----------------|----------------|----------------|----------------|
| Statistic\(^a\) | Sysmex UF-1000i result against urine culture | \(<10^4\) CFU/ml | \(10^4\)–\(10^5\) CFU/ml | \(\geq10^5\) CFU/ml |
| | \((n = 486 samples)\) | \((n = 26 samples)\) | \((n = 160 samples)\) | \((n = 31 samples)\) |
| Parametric Mean | 73.2 | 118.6 | 1,176.1 | 329.9 |
| 95% CI | 25.8–120.5 | 61.3–298.5 | 553.8–1,798.5 | 49.6–610.2 |
| SD | 531.2 | 445.3 | 3,985.7 | 764.2 |
| Nonparametric Median | 6.5 | 8.3 | 117.4 | 22 |
| 95% CI | 5.6–8 | 4.7–33.8 | 57.4–216.7 | 8.5–88.8 |
| Minimum | 0.0 | 1.5 | 0.8 | 4.1 |
| Maximum | 10,619.8 | 2,278.3 | 30,195.4 | 2,833.5 |
| IQR | 21.3 | 31.5 | 582.8 | 191.8 |

\(^a\) For leukocyte count by the Sysmex UF-1000i.
the corresponding ROC curve is shifted to the left. The area under the ROC curve is a key parameter for evaluating the performance of a test as a measure of accuracy and is independent of prevalence (“pure accuracy”). The point on the ROC curve nearest the upper left corner represents the best compromise between sensitivity and specificity (3, 6).

Among the 703 urine specimens submitted for culture, 186 (26.5%) were positive, 486 (69.1%) were negative, and 31 (4.4%) were contaminated. In the samples examined, we did not find any yeast.

Figure 4 shows the distribution of the microorganisms isolated in 186 true-positive samples examined: 26 samples with bacterial counts of $10^4$ to $10^5$ CFU/ml (14%) and 160 samples with bacterial counts of $\geq 10^5$ CFU/ml (86%). The urine cultures classified as contaminated were omitted.

To identify the microorganisms, conventional biochemical tests and the Vitek 2 system (bioMérieux, France) were used.

In 26 samples with positive bacterial counts between $10^4$ and $10^5$ CFU/ml, we isolated individual colonies of Gram-negative bacteria (4 *E. coli* and 5 non-*E. coli* colonies) and Gram-
positive bacteria (10 *Enterococcus* spp. and 4 *S. agalactiae*) and 3 associations of bacteria (*E. coli* plus Gram-positive bacteria, Gram-positive plus Gram-positive bacteria, and Gram-positive plus Gram-negative bacteria).

For the 160 samples with bacterial counts of $\geq 10^5$ CFU/ml, we isolated individual bacterial colonies of Gram-negative bacteria (79 *E. coli* and 27 non-*E. coli* colonies), Gram-positive bacteria (10 *Enterococcus* spp., 4 *S. agalactiae*, and 4 *S. aureus*), 33 associations of bacteria (6 *E. coli* plus Gram-positive bacteria, 15 associations of two Gram-negative bacteria, 9 associations of Gram-negative plus *Staphylococcus* spp., and 2 combinations of two Gram-positive bacteria) and 3 samples with lactobacilli.

A correlation analysis using Cohen’s $K$ between the two methods used was also performed. The coefficient $K$ measures the degree of agreement between two variables, in our case, the cutoff of positivity for bacteria and leukocytes. It presents a maximum of 1 when the agreement between two variables is perfect, 0 when agreement is no better than chance, and negative values when agreement is worse than chance $(4, 14)$. We found a value for coefficient $K$ of 0.49 ($P < 0.0001$).

**DISCUSSION**

The laboratory diagnosis of UTI is based on the detection and quantification of bacteria and leukocytes in urine. The presence of bacteria in the urine does not necessarily diagnose a UTI, as bacteriuria may also result from contamination of the sample or from normal bacterial colonization of the

![ROC curves of bacterial and WBC counts on the Sysmex UF-1000i flow cytometer.](image-url)
urethra. The presence of urinary leukocytes is often associated with UTI but may also derive from vaginal contamination in women.

We set the cutoff for positive urine culture at a bacterial count of \( \geq 10^5 \) CFU/ml, although in the literature, a bacterial count of \( \geq 10^4 \) CFU/ml is strongly associated with the presence of UTI for a sample collected using the technique of midstream collection (9, 11, 13). We set a lower cutoff with the aim of including infections from patients with permanent catheters or infections due to fastidious or slow-growing microorganisms.

Usually, a screening test is used for testing a population with a low prevalence of positivity. In our case, the prevalence of positive urine cultures was 30.8%. Thus, an ideal screening test might be able to reduce the number of samples examined by urine culture by about 70%.

However, the distribution of our results in Table 3 shows variable amounts of correct or incorrect results compared to cultured samples at different cutoffs of bacteriuria, with leukocyturia set at 100 WBC/ml. The sensitivity of the Sysmex UF-1000i increases at low values of bacteriuria, although some FN samples remain. At higher levels of bacteriuria, despite a loss of sensitivity and an increase in FN, PPV, and DA, the specificity of the Sysmex UF-1000i increases. Each laboratory may select lower cutoff values for bacterial counts to get better sensitivity or choose lower cutoff values flexibly for their own purposes to screen UTI and to minimize urine cultures.

The statistical analysis suggested 65 bacteria/ml and 100 WBC/ml as cutoff values. The combination of these values improved the performance of the screening process and allowed a reduction of 43% (302 samples) in bacterial culture while maintaining an acceptable level of FN (4 samples; 0.6%).

The FN were represented by \( E. coli \) isolates, all with a bacterial count between \( 10^4 \) and \( 10^5 \) CFU/ml. Moreover, these microorganisms were isolated from catheterized male patients aged between 75 and 94 years for whom the Sysmex UF-1000i indicated values for leukocytes of \(<34\) WBC/ml and values for bacteriuria between 13 and 43 bacteria/ml.

We set a cutoff value for bacteriuria of \( \geq 70 \) bacteria/ml for the above-mentioned microorganisms and for \( Lactobacillus \), which is considered a vaginal contaminant and therefore is not considered a pathological microorganism.

There remained a considerable number of FP (184 samples; 26%), as shown by the index of correlation, \( K \).

Cohen’s \( K \) index was statistically significant even if the value was not particularly high. This is supported by the fact that 184 cases were classified as FP, i.e., positive with the Sysmex UF-1000i but negative by urine culture.

On the other hand, a low \( K \) value is indicative of a paradoxical strength of the Sysmex UF-1000i, which is able to detect cases deemed negative by urine culture but significant for critically ill patients (i.e., catheterized patients).

We also found that the Sysmex UF-1000i is not very efficient in the detection of \( Enterococcus \) spp. at low loads. From a microbiological point of view, these bacteria grow at a very low rate on chromogenic agar, and thus, we might speculate that the dyes used by the flow cytometer are unable to penetrate the bacterial cell wall due to the development of a thickened wall or the formation of biofilms; indeed, these bacteria were found in catheterized patients.

Our study did not evaluate samples with yeast, as they were not found in the specimens that we analyzed. We usually report the presence of yeasts when isolated by urine culture, expressing the load qualitatively, i.e., as rare, discrete, or numerous colonies.

Yeasts are commonly members of the lower urinary tract flora, but they can have clinical relevance. In fact, hematogenous infection of the urinary tract is restricted to a few relatively uncommon microorganisms, such as \( S. aureus \), \( Candida \) spp., \( Salmonella \) spp., and \( Mycobacterium tuberculosis \), which cause primary infections elsewhere in the body.

\( Candida albicans \) readily causes a clinical UTI via the hematogenous route but is also an infrequent cause of an ascending infection if an indwelling catheter is present or following antibiotic therapy (9).

\( Candida \) infections can occur in any immunosuppressed patient but are more common in diabetic patients and those with chronic residual urine and where there is an indwelling catheter or stent. It is wise to treat all patients, even when they are asymptomatic, with antifungal agents. Removal of the catheter or stents is usually necessary (9).

Candiduria may clear spontaneously or may result in (or from) deep fungal infections. The presence of \( Candida \) species in the urine usually represents colonization and not infection,
and as such, not all patients with candiduria require treatment. Thus, an analysis with the Sysmex UF-1000i that is more focused on yeasts will be required.

This study shows that the routine screening of bacteriuria is useful in recognizing urinary tract infections and, secondly, that the cutoffs set for the Sysmex UF-1000i in the present study allowed a reduction in culture tests of 43%. These results are important, because they allowed a reduction in urine culture costs and freed up laboratory resources for other activities.

When urine specimens are submitted for culture, generally one CPS agar plate is used to test for bacterial growth. Additional plates may also be necessary (Columbia CNA agar plus 5% ram’s blood and/or MacConkey agar plates) when cultures are positive for two different microorganisms, and further costs are incurred. The Sysmex UF-1000i performs the urinalysis and urine culture using the same sample.

The advantage of using the Sysmex UF-1000i versus the culture assay is not only the reduced number of samples to be put on culture, but also the rapidity in identifying the negative samples, higher specificity, and maximum recovery of FN with minimization of FP.

The cutoff of 10^4 CFU/ml implies that a larger number of samples must be cultured but avoids the loss of critical patients for whom a bacterial count of 10^4 CFU/ml might be significant.

The next step is to optimize the Sysmex UF-1000i by setting a cutoff of bacteriuria personalized for each patient, i.e., by sex, age, and presence of a catheter, or for urological patients, in accordance with recent British and European guidelines for interpretation of microbiological urine culture (9, 13).

For example, in the Scottish Intercollegiate Guidelines Network (SIGN) guidelines, it was reported that symptomatic bacteriuria occurs in 17 to 20% of pregnancies, and there are pathophysiological grounds to support a link to prelabour, premature rupture of membranes (PPROM), and preterm labor. Untreated upper urinary tract infection in pregnancy also carries well-documented risks of morbidity and, rarely, mortality for the pregnant woman.

Two to 9% of pregnant women are bacteriuric in the first trimester, a prevalence similar to that in nonpregnant women of the same age; 10 to 30% of women with bacteriuria in the first trimester develop upper urinary tract infection in the second or third trimester. In these patients, it is important to consider even low counts of Gram-positive bacteria (in particular, S. agalactiae), which do not cause upper UTI but are implicated in causing premature delivery (13). This positivity is often associated with a false-negative dipstick test.

The cutoff setting is important for some specific groups of patients (i.e., pregnant women, catheterized patients, and urological patients) with significant bacteriuria. In fact, for these patients, a bacterial count of ≤10^4 CFU/ml is important (13). In children, when the urine is obtained by bladder catheterization, the urine culture is considered positive with more than 10^5 CFU/ml, while if the urine specimen is collected from midstream void, it is considered positive with ≥10^5 CFU/ml in patients with symptoms and ≥10^4 CFU/ml in patients without symptoms (11).

Our study demonstrates that the automatic determination of leukocyturia and bacteriuria can be routinely applicable. Automatic systems, although still open to improvement, allow savings in unnecessary urine culture, enable clinical decisions to be made more quickly upon receipt of negative results, and, where possible, limit the number of FP with a marginal share of FN results.

Optimization for each patient may improve the effectiveness of microbiological diagnosis, freeing resources for activities requiring greater professional input. The choice to use automatic systems should be considered and applied in specific contexts with the aim of optimizing laboratory efficiency.

In fact, bacterial results of urine culture are usually provided more than 2 days after collection (culture results in 18 to 24 h and susceptibility tests after 24 h), and the diagnosis of UTI is performed on the basis of symptoms and, sometimes, urinalysis. In many cases, when physicians suspect that a patient has UTI by symptoms and signs, empirical antibiotics are prescribed without confirmative culture results, and patients may receive unnecessary antibiotics. By predicting the results of urine culture with the Sysmex UF-1000i, physicians who suspect UTI can obtain more information, and the unnecessary prescription of antibiotics can be reduced. Also, workloads and laboratory costs can be lowered. The main advantage, however, is the possibility to report negative results in real time.

In summary, the Sysmex UF-1000i is an ideal device for performing accurate enumeration of bacterial cells in urine and for detecting significant bacteriuria in a short time. The bacterial counts generated by Sysmex UF-1000i analysis may be useful for screening to exclude UTI and may contribute to the reduction of unnecessary urine cultures and empirical antibiotic prescriptions.

For a screening method, the time needed to perform the analysis is another important factor. The Sysmex UF-1000i can perform quantitative urinalysis, including detection of significant bacteriuria, and provide a full report within 60 s. Physicians can obtain information on the probability of a positive culture very quickly, and unnecessary urine cultures can be avoided. Consideration of patient symptoms in combination with urinalysis results and detection of significant bacteriuria with the Sysmex UF-1000i can assist in clinical decision making regarding the initiation of treatment for urinary tract infection.

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REFERENCES