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Use of flow cytometry (Sysmex® UF-100) to screen for positive urine cultures: in search for the ideal cut-off

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Abstract

Background: Cultures for urinary tract infections (UTI) constitute a large workload in the clinical microbiology laboratory, although up to 80% are usually negative. Several automated methods are available to screen urines for UTI, one being the flow cytometry-based Sysmex® UF-100.

Methods: The performance of the UF-100 was evaluated over a 16-month period using urine culture as the reference method.

Results: During this period, a total of 5356 urine samples were studied (469 children; 3229 women and 1658 men), of which 706 were culture positive (593 grew Gram negative bacilli). Receiver operating characteristics (ROC) curve analysis showed an area under the curve (AUC) of 0.83 for leukocytes and 0.85 for bacterial count. Applying cut-off values reported in the literature gave sensitivities ranging from 75% to 90%, resulting in 73–174 false negatives (FN). Using a logical combination (leukocytes $\geq 15\times10^6$/L OR bacteria $\geq 500\times10^6$/L) gave a sensitivity of 98%. However, the specificity dropped to 25%, resulting in 15 FN.

Conclusions: Screening urine samples for UTI detects a large number of culture positive samples. However, the rather large number of FN observed precludes the use of the UF-100 as a routine screening method to exclude urine samples from culture.

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Keywords: flow cytometric screening; Sysmex® UF-100; urine culture.

Introduction

Urine culture is of the most frequent culture analysis performed in the clinical microbiology laboratory. However, 70%–80% of cultures are negative (1, 2). In the author's institution, ~26,000 urine cultures were performed during 2008, 19% of which were culture positive. Negative urine cultures lead to considerable workload and consumption of resources, an important aspect in times of ever increasing cost restrains. This has led to the development and implementation of screening methods to identify urinary tract infections (UTI). One of the first automated methods for urine sediment testing was based on image analysis (3, 4). However, these systems still require an operator to visually inspect images of cells and casts (5). Around the same time, improved urinary test strips and instruments for reading them, were being developed (6). Screening for UTI was based on the detection of nitrite and leukocyte esterase (7, 8). However, false negative (FN) and/or false positive results were too frequent, precluding use of this approach as a screening method for UTI (9, 10).

Recent approaches based on flow cytometry allow detection and counting of large numbers of particles in a short period of time, including bacteria and yeast (6). One such instrument for this purpose is the Sysmex® UF-100. Several studies reported acceptable performance for this instrument (6, 11–13). However, reported results for sensitivity and specificity varied significantly, with some showing a rather high number of FN results (1, 2). For example, some studies used higher cut offs, e.g., 300×10^7 bacteria/L (11, 12), in an attempt to increase the specificity to >60%, while maintaining sensitivity above 90%. However, it appears that most or all of the samples were not from hospital acquired UTIs (11, 12).

The goal of this study was to evaluate the Sysmex® UF-100 system to screen urine for UTI under routine conditions in an unselected population at a major tertiary referral centre.

Materials and methods

The study was performed and approved within the ethical framework of the hospital. The study includes data from inpatients and outpatients that had a urine culture and a flow cytometric urine screen (Sysmex® UF-100, TOA Medical Electronics/Europe GmbH, Hamburg, Germany) performed between January 2008 and June 2009. Urine was collected into a Urine-Monovette® (Sarstedt, Nürmbrecht, Germany) containing boric acid for urine culture, while Urine-Monovette® tubes without boric acid were used for the flow cytometric and dipstick analysis. Only those samples were considered where both Monovette® tubes were logged into the Laboratory Information System of the Clinical Chemistry and Microbiology Department <1 h apart.

Dipstick urinalysis was performed using the automated Urisys® 2400 (Roche Diagnostic Systems, Lisbon, Portugal).

Manual microscopy sediment analysis was performed according to CLSI GP 16-A guideline (14). Each sample was centrifuged at 400 *g* for 10 min, the supernatant was decanted and the sediment mixed in the residual volume. At a 400× magnification, 20 random

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microscopic fields were examined and the mean number of cells per field were calculated.

Urine cultures were performed by inoculating well mixed, uncentrifuged urine on horse blood agar and MacConkey agar plates (bioMérieux, Lisbon, Portugal). Growth was assessed after overnight incubation at 36°C. Urine cultures were considered negative if no growth occurred or with growth $<10^6$ CFU/L. If more than two types of bacteria grew, samples were considered contaminated. If growth of a pure culture occurred, samples were considered positive in the presence of leukocyturia (at least 5 leukocytes/microscopic field). However, in certain patient groups, such as children, immunocompromised, transplant recipients or pregnant women, growth of a pure culture was considered positive, even in the case of <5 leukocytes/microscopic field. Microorganisms were identified by conventional biochemical tests using the WalkAway®96 system (Dade Behring, Marburg GmbH, Germany) and/or the VITEK system (bioMérieux, Lisbon, Portugal).

For flow cytomeric urine screening, the Sysmex® UF-100 was used. This system analyzes formed elements in urine, including cells, based on flow cytometry and impedance detection as described previously (15, 16). In cases where patients had multiple samples processed, only the first sample was considered. Samples that were considered to represent bacterial contamination were excluded. The culture results were used as the reference method to evaluate the performance of the Sysmex® UF-100 as a screening method for UTI.

Data were analyzed using SPSS Statistics V17.0 (SPSS Inc, Chicago, IL, USA).

Results

A total of 5356 (4650 culture negative and 706 culture positive) urine samples fullfield the inclusion criteria. Of these, 469 were from children <16 years (mean: 6 years, range: 2 days–15 years), 3229 from female patients (mean: 50 years, range: 16–100 years) and 1658 from male patients (mean: 62 years, range: 16–97 years). Bacteria that were isolated are shown in Table 1.

Receiver operating characteristics (ROC) curve analysis (Figure 1) showed an area under the curve (AUC) of 0.83 and 0.85 for leukocytes and bacterial counts, respectively. Results obtained at different cut-off thresholds are shown in Table 2.

The best cut-off was the combination leukocytes $\geq 15\times10^6$/L OR Bacteria $\geq 500\times10^6$/L, which produced a sensitivity of 98%, but a specificity of only 25%, resulting in 15 FN. Of these FN, two were from Obstetrics and three from the Nephrology Department. Underlying conditions most frequently associated with FN were renal transplantation (n=2), pregnancy (n=2), urinary tract structural problems (n=4), immunosuppression (n=2) and antibiotherapy (n=2).

Discussion

Urine cultures constitute a major part of the clinical microbiology workload. Considering that 70%–80% of urine cultures are usually negative (1, 2), a screening test that reliably identifies samples from patients with a UTI would have a major impact, and large cost savings could be achieved. The appearance of flow cytometric urine analysis seemed to provide such a method. However, such a screening method would need to perform well in uncomplicated UTIs. Many cases of uncomplicated UTIs, for example in young, sexually active women, are likely treated empirically, without resorting to urine culture (17). However, complicated UTIs often associated with complex underlying pathologies, such as immunosuppression, are often caused by hospital-related, antibiotic resistant strains (17). For these infections, a screening tool for UTI would have to offer reliable detection when used in a large hospital setting. In this context, it is notable that some of the studies that evaluated the Sysmex® UF-100 excluded hospital acquired infections (12).

Table 1 Bacterial isolates from positive urine cultures (n=706).

Bacteria	Children (<16 years)	Adult, male	Adult, female
Gram negative bacilli	49	140	404
Escherichia coli	26	78	287
Proteus mirabilis	7	11	40
Pseudomonas spp.	2	17	18
Klebsiella spp.	8	19	44
Other	6	15	15
Gram positive cocci	10	47	56
Group B *Streptococcus*	0	3	23
Enterococcus spp.	7	25	22
Staphylococcus spp.	3	19	11
Total	59	187	460

Other=*Morganella morganii*; *Serratia* spp.; *Citrobacter* spp.; *Enterobacter* spp.; *Providencia* spp.; *Stenotrophomonas maltophilia*.

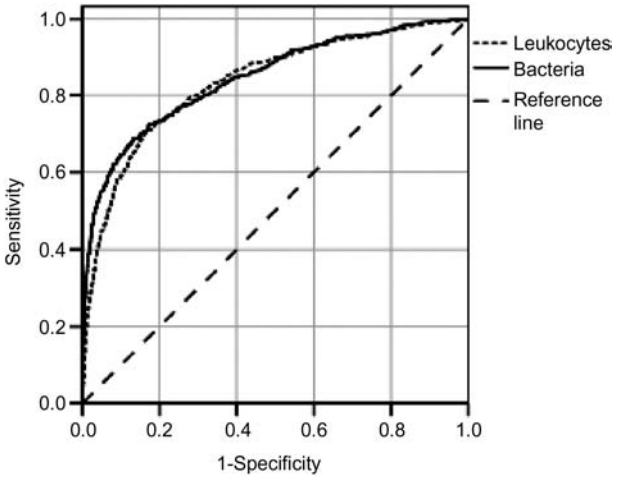

Figure 1 ROC curve for leukocyte and bacterial counts (n=5356). Receiver operating characteristics (ROC) curve for bacteria and leukocyte counts on the Sysmex® UF-100 flow cytometer, using urine culture as the reference method. The area under the curve (AUC) is 0.83 for leukocytes and 0.85 for bacterial counts (0.5–0.7: low accuracy, 0.7–0.9: moderate accuracy and >0.9 high accuracy). ROC curve generated with SPSS Statistics V17.0.

Table 2 Performance of the Sysmex® UF-100 at different cut-off thresholds for leukocyte and bacteria counts.

Cut-off	Sensitivity, %	Specificity, %	False negatives, n	Negative cultures avoided/year[a]
Leukocytes $\geq 15\times10^6$/L	87	59	93	12,425
Leukocytes $\geq 20\times10^6$/L	84	65	115	13,689
Leukocytes $\geq 30\times10^9$/L	79	73	14	15,374
Bacteria $\geq 600\times10^6$/L	94	36	41	7582
Bacteria $\geq 150\times10^7$/L[12]	88	58	85	12,180
Bacteria $\geq 180\times10^7$/L[b]	83	63	121	13,268
Bacteria $\geq 200\times10^7$/L	81	66	135	13,900
Bacteria $> 300\times10^7$/L[12]	75	77	174	16,216
Bacteria $> 380\times10^7$/L[18]	71	85	179	17,850
Leukocytes $\geq 15\times10^6$/L OR Bacteria $\geq 500\times10^6$/L[c]	**98**	**25**	**15**	**5265**
Leukocytes $\geq 30\times10^6$/L OR Bacteria $\geq 600\times10^6$/L	97	31	23	6529
Leukocytes $\geq 111\times10^6$/L OR Bacteria $> 300\times10^7$/L[11]	85	73	107	15,271
Bacteria $> 180\times10^7$/L OR Leukocytes $> 45\times10^6$/L[15]	90	57	73	12,004
Leukocytes $\geq 30\times10^6$/L OR Bacteria $\geq 300\times10^7$/L OR Nitrites positive	88	65	70	13,689

11, 12, 15, 18 = References of previous studies from which the cut-off was applied. Sample, (n) = 5356 (4650 culture negative and 706 culture positive); [a]Estimation of the number of urine cultures that be avoided per year based on the assumption that 26,000 cultures are performed, of which 81% are culture negative (21,060). [b]Manufacturer's reference cut-off. [c]Best value for sensitivity. Values in bold indicate the cut-off that gave best results in terms of sensitivity/specificity.

Although the Sysmex® UF-100 detects a large percentage of UTIs, reported sensitivities are only in the range of 70%–90% (1, 2, 15, 18). However, some investigators reported higher sensitivities (>95%), but this caused the specificity to drop to values as low as 15%–49% (11, 12). Interestingly, the cut offs that were used in these studies varied widely (Table 2) (11, 12, 15, 18). When these cut offs were applied in the current study, the best sensitivity did not exceed 90%, with a rather low specificity of 57%. To achieve the best cut-off, a logical combination of a low leukocyte count ($>15\times10^6$/L) and bacterial count (500×10^6/L) was used (Table 2). However, this resulted in a large number of false positives as the specificity decreased to 25%. Extrapolating these results to 26,000 urine cultures/year that our laboratory processes, with a 19% positive rate, translates into 99 FN (2%) and 5265 negative urine cultures that would be avoided. Interestingly, some studies used 300×10^6 bacteria/L as the cut-off, with good results for sensitivity (~95%) and specificity (>60%) (11, 12). However, these studies included a much smaller number of cultures. For example, Kim et al. included 330 urine cultures and excluded hospital acquired infections (12). In addition, one study reported 16 FN out of a total of 427 positive cultures (11). This illustrates the importance of investigating the usefulness of this method for screening for all types of UTIs.

It has already been reported that most FN results are observed primarily in certain patient groups, including those that are immunosuppressed, renal transplant recipients or pregnant woman (2). Of course, it is possible that in some of these cases, microbiologists ''over'' interpret the urine culture and consider them significant. However, rigorous application of criteria, such as the leukocyte count or bacterial growth would not support this interpretation. Such urine specimens most likely would show a negative screening result using the Sysmex® UF-100. Still, one could use the screening method to exclude unnecessary urine culture specimens, applying a high cut-off sensitivity of 98% or 99%, and accepting low specificity. For example, applying a sensitivity of 98% in the authors' hospital, 5364 cultures could have been avoided in 2008. Even so, this approach would have missed 99 positive urine cultures (FN), an unacceptably high number.

In conclusion, the Sysmex® UF-100 is a useful method for screening urine samples for UTI. However, clear cut-off values are not readily available and are difficult to establish. For this method to be a useful screening tool for UTI in the hospital setting, the FN rate would have to be acceptably low. This, possibly, would require instrument performance with sensitivity >99% to ensure that clinically important cases of UTI would not be missed.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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References

1. Okada H, Yutaka S, Miyazaki S, Arakawa S, Hamagushi Y, Kamidono S. Detection of significant bacteriuria by automated urinalysis using flow cytometry. J Clin Microbiol 2000;38: 2870–2.
2. Zaman Z, Roggeman S, Verhaegen J. Unsatisfactory performance of flow cytometer UF-100 and urine strips in predicting outcome of urine cultures. J Clin Microbiol 2001;39:4169–71.
3. Deindorfer FH, Gangwer JR, Laird CW, Ringold RR. ''The Yellow IRIS'' urinalysis workstation – the first commercial application of ''automated intelligent microscopy''. Clin Chem 1985;31:1491–9.
4. Roe CE, Carlson DA, Daigneault RW, Statland BE. Evaluation of the Yellow IRIS: an automated method for urinalysis. Am J Clin Path 1986;20:436–9.
5. Bem-Erza J, Bork L, McPherson RA. Evaluation of the Sysmex UF-100 automated urianalysis analyzer. Clin Chem 1998;44: 92–5.
6. Hanneman-Pohl K, Kampf SC. Automation of urine sediment examination: a comparison of the Sysmex UF-100 automated flow cytometer with routine manual diagnosis (microscopy, test strips and bacterial culture). Clin Chem Lab Med 1999;37: 753–64.
7. Koening C, Tick L, Hanna B. Analyses of the dflash track DNA probe and UTI screen bioluminescence tests for bacteriuria. J Clin Microbiol 1992;30:342–5.
8. Pfaller M, Koontz P. Laboratory evaluation of leukocyte esterase and nitrite test for detection of bacteriuria. J Clin Microbiol 1985;21:840–2.
9. Sawyer K, Stone L. Evaluation of a leukocyte dipstick test used for screening urine cultures. J Clin Microbiol 1984;23:160–2.
10. Jones C, McPherson D, Stevenson D. Inability of the Chemstrip LN compared with quantitative urine culture to predict significant bacteriuria. J Clin Microbiol 1986;23:160–2.
11. Evans R, Davinson MM, Sim LRW, Hay AJ. Testing by Sysmex UF-100 flow cytometer and with bacterial culture in a diagnostic laboratory: a comparison. J Clin Path 2006;59: 661–2.
12. Kim SY, Kim YJ, Lee SM, Hwang SH, Kim HH, Lee HY. Evaluation of Sysmex UF-100 urine cell analyzer as a screening test to reduce the need for urine cultures for community-acquired urinary tract infections. Am J Clin Path 2007;128: 922–5.
13. Manoni F, Valverde S, Antico F, Salvadego MM, Giacomini A, Gessoni G. Field evaluation of a second-generation flow cytometer UF-100 in diagnosis of acute urinary tract infections in adult patients. Clin Microbiol Infect 2002;8:662–8.
14. CLSI. Routine urinalysis and collection, transportation, and preservation of urine specimens; approved guideline. CLSI document GP16-A3. CLSI, Wayne Pennsylvania, USA, 2001.
15. Fenili D, Pirovano B. The automation of sediment urinalysis using a new urine flow cytometer (UF-100™). Clin Chem Lab Med 1998;36:909–17.
16. Keijze MH, Brandts RW. Flow cytometry and the urine laboratory: field evaluation of Sysmex UF-100™. Sysmex J Int 1997;7:117–22.
17. Stamm WE, Hooton TM. Management of urinary tract infections in adults. N Engl J Med 1993;329:1328–34.
18. Koken T, Aktepe OC, Serteser M, Samli M, Kahraman A, Dogan N. Determination of cut-off values for leucocytes and bacteria for urine flow cytometer (UF-100) in urinary tract infections. Int Urol Nephrol 2002;34:175–8.