Comparison of Sysmex UF-100 and UF-1000i with urine culture for the diagnosis of urinary tract infections

Fabio Manoni[1], Agostino Tinello[1], Lucia Fornasiero[1], Paolo Hoffer[1], Sara Valverde[2], Gianluca Gessoni[2]
[1]Servizio di Medicina di Laboratorio, Ospedale di Monselice, PD
[2]Servizio di Medicina di Laboratorio, Ospedale Civile di Chioggia, VE

ABSTRACT

Urinary tract infections (UTI) are a common clinical condition. The gold standard for diagnosis is still the bacterial culture, even though a large proportion of evaluated samples are negative. Unnecessary cultures can be reduced by an effective screening test. The aim of this study was to compare two cytometers for rapid diagnosis of UTI. Using 209 urine samples submitted to our laboratory for microbiological examination, we evaluated the analytical performance of the new urine cytometer UF-1000i in comparison with the previous generation analyzer UF-100 (both from Sysmex). We compared bacteria (BACT) and leukocyte (WBC) counts performed by UF-1000i and UF-100 with colony-forming units (CFU) quantification on citrate lactose electrolytes deficient (CLED) agar to assess the best cut-off values. Moreover, a correlation between BACT and WBC quantification performed by the two instruments was carried out. In comparison with $1x10^8$ CFU/L, cut-off values of $1.25x10^8$ BACT/L and $4x10^7$ WBC/L by using UF-1000i, and $3x10^9$ BACT/L and $3.5x10^7$ WBC/L by using UF-100 were obtained, respectively. While WBC quantification by UF-1000i and UF-100 showed a strong correlation (r=0.98), BACT quantification displayed a poor correlation (r=0.59).

INTRODUCTION

Urinary tract infections (UTI) are at the second place in the frequency of all causes of infection after respiratory ones. However, the request for microbiological examination of urine samples exceeds that for detection of respiratory pathogens, making urine culture the most common microbiologic test in diagnostic laboratories.

The presence of microorganisms alone in a urine specimen is not significant in itself because a high level of bacteriuria can occur from colonisation and contamination as well as infection. The measurement of pyuria is the most readily available way to detect a host response, thus differentiating colonization and contamination from infection (1).

The presence of a single microorganism at a concentration of almost $1x10^8$ colony forming units (CFU)/L in voided urine specimens is the generally accepted definition of significant bacteriuria (2), but lower limits, such as $1x10^7$ CFU/L, were suggested for children, men and patients with underlying diseases or for fastidious microorganism (3). The presence of associated pyuria is a good predictor of bladder infections, even though there is not agreement about the cut-off value for considering pyuria as "significant".

We previously reported evaluations of Sysmex UF-100 and UF-1000i in rapid diagnosis of UTI (4, 5). In this study, we performed an evaluation of these two analyzers, using the same samples, in comparison with quantitative urine culture, with the aim to provide information that could be useful in the technology assessment process.

MATERIALS AND METHODS

Samples

We considered 209 consecutive urine samples, obtained from adult patients with voided midstream technique, submitted in a week to our Clinical Pathology Laboratory for a microbiologic evaluation for a suspected UTI. All samples were collected in a sterile 100 mL device. By using a vacuum system that reduces the manipulation of the sample (Vacutainer, Becton Dickinson), from the primary collection container of each subject, 3 tubes were obtained: one for microbiologic, one for UF-100 and one for UF-1000i examination.

Microbiologic analysis

The quantitative urine culture consists of the inoculation of 1 µL urine into a citrate lactose electrolytes deficient (CLED) agar plate using a sterile calibrated

Correspondence to: Gianluca Gessoni, Transfusional Service, Ospedale Civile, Via Madonna Marina 500, 30015 Chioggia (VE). Tel. 0415534400, Fax 0415534401, E-mail ggessoni@asl14chioggia.veneto.it

Ricevuto: 21.11.2011 Revisionato: 19.12.2011 Accettato: 21.12.2011

loop. Following an incubation for 24 h at 37 °C, the number of colonies is counted and multiplied by 10^3 to give an estimate of the number of microorganisms/mL. We adopted selective and differential media: McConkey agar to obtain a better demonstration of Enterobacteriaceae and columbia nalixic acid agar (CNA) to obtain a better demonstration of group D Enterococci. In cultures with a significant bacteriuria, a biochemical identification and an investigation of the sensitivity to antimicrobial drugs was performed using the Phoenix automated system (Becton Dickinson). Antimicrobial susceptibility tests were a miniaturisation of the broth dilution susceptibility test. Conventional and chromogenic tests were adopted for biochemical identification of urinary bacteria (BACT).

Flow cytometry analysis

All samples were tested within 2 h from collection by using Sysmex UF-100 and UF-1000i analysers.

UF-100 is an automated analyser that performs urinalysis with flow cytometry. The system aspirates 0.8 mL of urine and performs the analysis of cells [erythrocyte, leukocytes (WBC) and epithelial cells], BACT and casts by using electrical impedance for volume, forwards light scatter for size and uses a couple of fluorescent dyes for nuclear and cytoplasmic characteristics. The formed elements are categorised in a two-dimensional space (scattergrams) on the basis of their size, shape, volume, and staining characteristics.

UF-1000i combines flow cytometry with impedance analysis of urine after staining with two polymethine dyes. The instrument can identify red blood cells, WBC, squamous epithelial cells, small round cells, hyaline casts, pathologic casts, BACT, yeast-like cells, spermatozoa, and crystals. UF-1000i has a separate analytic channel with a dedicated reagent system for BACT-specific staining, allowing a very sensitive BACT detection, down to concentration levels between 10^5 and 10^6 BACT/L.

Statistical analysis

Quantitative measures, such as CFU quantification, BACT and WBC counts, were related by using linear regression statistics and Bland-Altman plots. Moreover, the following statistical parameters were considered: median value with 95% confidence interval (CI), interquartile range (IQR), sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and correctly classified incidence (CCI). To assess the best cut-off values, we plotted the ROC curves. Statistical analysis was performed with the software program Analyse.it, version 2.03.

RESULTS

Culture results

By using quantitative culture results, the presence of $1x10^8$ CFU/L was considered diagnostic for UTI. Among the 209 patients evaluated in this study, 78 (37%) showed a bacteriuria >10^8 CFU/L (pre-test probability, 0.37).

Comparison between culture and UF-100

By using the UF-100 analyzer, the median BACT count was $1.7x10^8$ BACT/L in negative samples and $42.2 x10^8$ BACT/L in positive samples, respectively. The median WBC count was $0.6x10^7$ WBC/L in negative samples and $13.9x10^7$ WBC/L in positive samples, respectively. Quantification of BACT and WBC obtained by using UF-100 analyzer in samples positive and negative at microbiologic examination is reported in Figure 1; Kruskal-Wallis statistical test showed a highly significant difference (P <0.001) both for BACT and WBC counts.

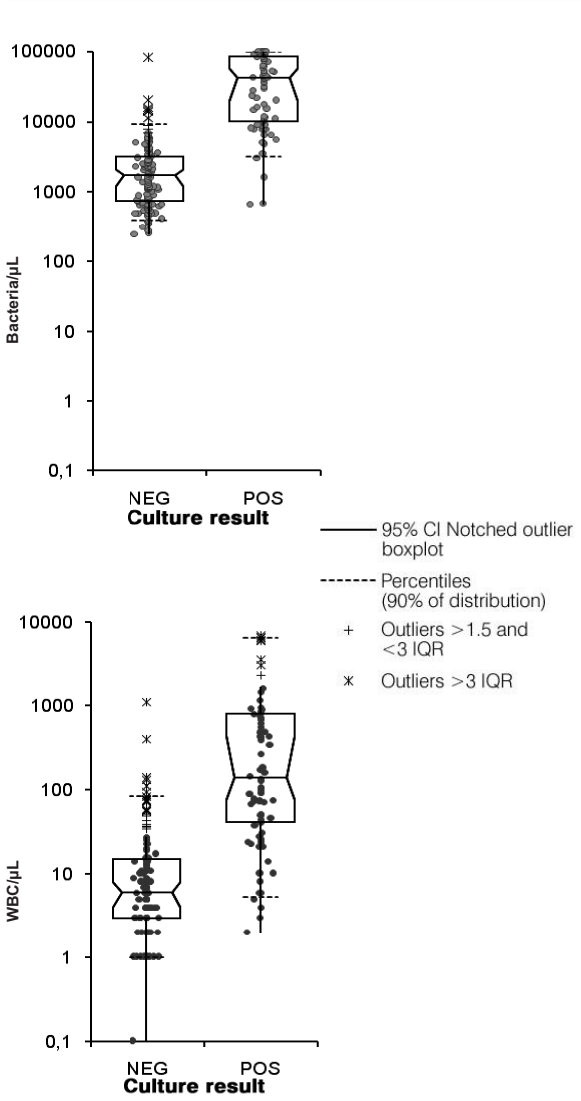

Figure 1
Bacteria and leukocyte (WBC) quantification performed in positive and negative urine culture samples by using the UF-100 system. IQR, interquartile range.

For BACT, the area under the ROC curve was 0.95. Using the curve, the best cut-off value was $3x10^9$ BACT/L. By using this cut-off value, we observed a SE of 0.96 (CI: 0.89 to 0.99), a SP of 0.74 (CI: 0.66 to 0.81), a PPV of 0.69 (CI: 0.59 to 0.77), a NPV of 0.97 (CI: 0.92 to 0.99), and a CCI of 0.82 (CI: 0.76 to 0.88). The best cut-off value established by using UF-100 analyser for WBC was $3.5x10^7$ WBC/L, with an area under the ROC curve of 0.89. By using this cut-off value, we observed a SE of 0.88 (CI: 0.78 to 0.94), a SP of 0.81 (CI: 0.72 to 0.87), a PPV of 0.72 (CI: 0.62 to 0.82), a NPV of 0.91 (CI: 0.84 to 0.96), and a CCI of 0.83 (CI: 0.77 to 0.88). Table 1 reports a summary of data.

Comparison between culture and UF-1000i

By using the UF-1000i analyzer, the median BACT count was $1.8x10^7$ BACT/L in negative samples and $6.1 x10^8$ BACT/L in positive samples, respectively. The median WBC count was $0.5x10^7$ WBC/L in negative samples and $19.2x10^7$ WBC/L in positive samples, respectively. Quantification of BACT and WBC obtained by using UF-1000i analyzer in samples positive and negative at microbiologic examination is reported in Figure 2; Kruskal-Wallis statistical test showed a highly significant difference ($p < 0.0001$) both for BACT and WBC counts.

For BACT, the area under the ROC curve was 0.99. Using the curve, the best cut-off value was $1.25x10^8$ BACT/L. By using this cut-off value, we observed a SE of 0.97 (CI: 0.91 to 0.99), a SP of 0.94 (CI: 0.88 to 0.97), a PPV of 0.92 (CI: 0.83 to 0.96), a NPV of 0.98 (CI: 0.94 to 0.99), and a CCI of 0.96 (CI: 0.92 to 0.98). The best cut-off value established by using UF-1000i analyser for WBC was $4.0x10^7$ WBC/L, with an area under ROC

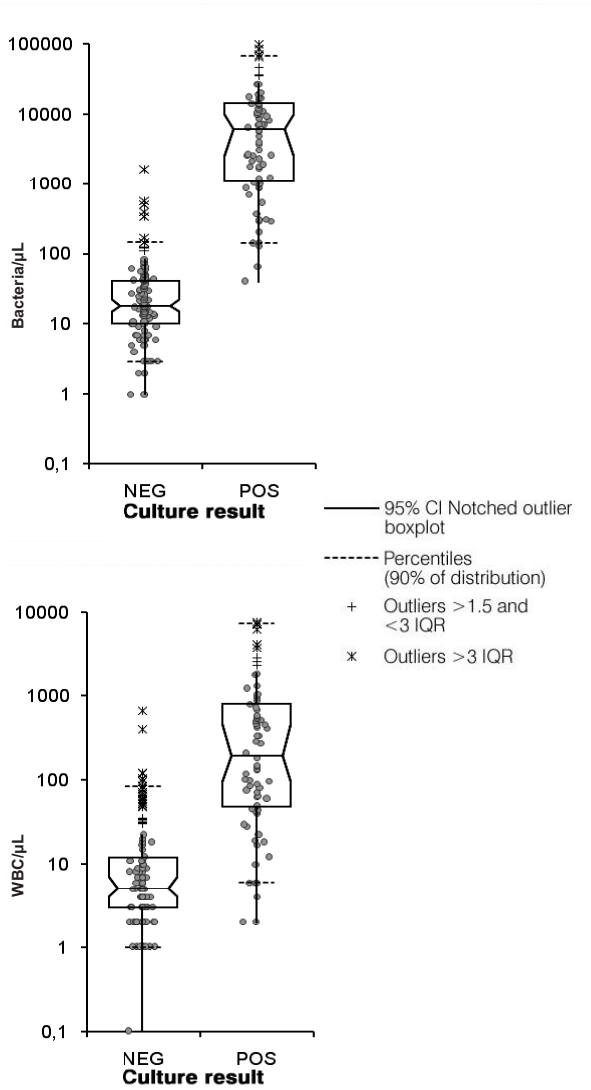

Figure 2
Bacteria and leukocyte (WBC) quantification performed in positive and negative urine culture samples by using the UF-1000i system. IQR, interquartile range.

Table 1
Performance of Sysmex UF-100 and UF-1000i for diagnosis of urinary tract infections in comparison with quantitative urine culture

	UF-100		
	BACT	WBC	BACT+WBC
SE	0.96	0.88	0.97
SP	0.74	0.81	0.72
PPV	0.69	0.72	0.73
NPV	0.97	0.91	0.97
CCI	0.82	0.83	0.81
	UF-1000i		
	BACT	WBC	BACT+WBC
SE	0.97	0.81	0.98
SP	0.94	0.86	0.80
PPV	0.92	0.78	0.81
NPV	0.98	0.88	0.98
CCI	0.96	0.84	0.85

BACT, urinary bacteria; WBC, urinary leukocytes; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; CCI, correctly classified incidence.

Table 2
Comparison of UF-100 and UF-1000i performances with urine culture results. Among 209 evaluated samples, 78 positive cultures were observed

	UF-100		UF-1000i	
	BACT	WBC	BACT	WBC
TP	75	68	76	63
FP	34	26	7	18
FN	3	10	2	15
TN	97	105	124	113

BACT, urinary bacteria; WBC, urinary leukocytes; TP, true positives; FP, false positives; FN, false negatives; TN, true negatives.

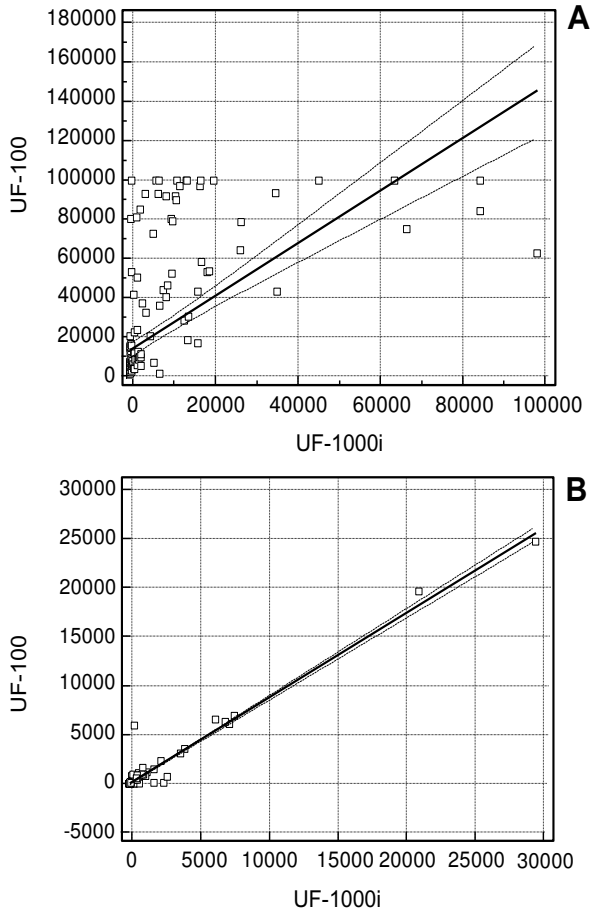

Figure 3
Correlations between UF-1000i and UF-100 analyzers. Plot A displays results for bacteria and plot B displays results for leukocytes.

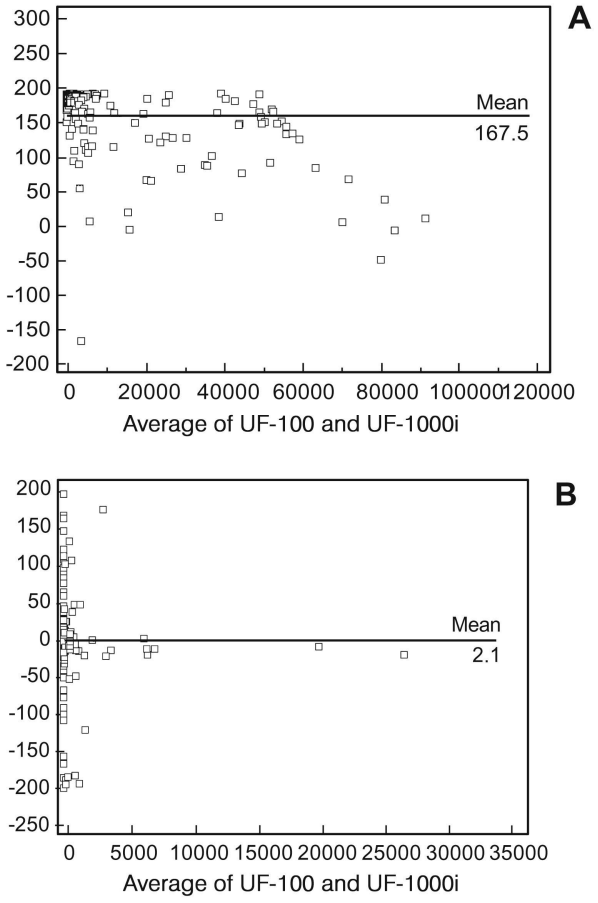

Figure 4
Bland-Altman plots of differences in percentage (y-axis) between UF-100 and UF-1000i analysers. Plot A displays results for bacteria and plot B displays results for leukocytes.

curve of 0.91. By using this cut-off value, we observed a SE of 0.81 (CI: 0.71 to 0.89), a SP of 0.86 (CI: 0.79 to 0.92), a PPV of 0.78 (CI: 0.67 to 0.86), a NPV of 0.88 (CI: 0.81 to 0.93), and a CCI of 0.84 (CI: 0.78 to 0.89). Data are summarized in Table 1.

Comparison between UF-100 and UF-1000i

Table 2 displays the performance of the two flow cytometers when compared with urine culture results.

By using linear regression statistics, we compared BACT and WBC quantification obtained with UF-100 and UF-1000i. While for BACT a relatively weak correlation (r=0.59, CI: 0.49-0.67) was observed, for WBC a strong correlation (r=0.98, CI: 0.97-0.99) was obtained. Correlation results are reported in Figure 3. Using Bland-Altman plots, we observed no significant differences in WBC counts, while an overestimation of BACT quantification performed by using UF-100 vs. UF-1000i was detected (Figure 4).

DISCUSSION

After the introduction of automated equipments for the analysis of insoluble urine components, various Authors focused their attention on the application of cytometry to quantification of BACT and WBC for diagnosis of UTI. Results obtained with Sysmex UF-100 analyzer, a second generation cytometer devoted to urine particles analysis, were often reported as satisfactory, even if some failures were also detected (5-12). Published cut-off values are between 1.6 and $3.4x10^7$ WBC/L and between 1.5 and $3.0x10^9$ BACT/L (5-12). Relatively few literature data are also available on the performance of UF-1000i, a third generation cytometer for urine particle analysis (4, 13-16). These data suggest for UF-1000i a lower cut-off value for BACT ($0.5\text{-}1.25x10^7$ BACT/L), with cut-off for WBC comparable with that reported using UF-100. Experimental cut-off values obtained in this study for UF-100 were $3x10^9$ BACT/L and $3.5x10^7$ WBC/L. These values are in good agreement with data reported in literature (5-12). By using UF-1000i, experimental cut-off values obtained in this study were $1.25x10^8$ BACT/L and $4x10^7$ WBC/L. Also in this case, values are in good agreement with literature data [4,13-17].

Both UF-100 and UF-1000i analyzers adopt similar

technical approaches to WBC quantification. These approaches are based upon a combination of flow cytometry with impedance analysis of urine after staining with fluorescent dyes, carbocyanine for cell membranes and phenanthridine for nucleic acids for UF-100, and new polymeric dyes stainings nucleic acids for UF-1000i. Despite of these differences in dyes, WBC quantification performed by using UF-100 and UF-1000i showed a strong correlation (P <0.001) and the Bland-Altman plot confirmed the absence of bias. Demonstration of significant pyuria is important to differentiate infections from colonization and contamination of urine. Moreover, pyuria with the presence of symptoms and the absence of bacterial growth on routine laboratory media, suggests an infection sustained by fastidious bacteria. The classical culture method needs 24 h for results, whereas Sysmex UF-100 and UF-1000i analyzers give results in a few minutes, thus reducing the microbiology turnaround time with obvious benefits for patients and physicians.

BACT quantification performed by UF-100 analyzer showed some problems, due to the high background signals requiring the adoption of higher cut-off values to improve result specificity. In this study, after plotting the ROC curve, a cut-off value of $3x10^9$ BACT/L was obtained. This result is about tenfold higher than cut-off value adopted for cultural examination (10^8 CFU/L). UF-1000i has a separate analytical channel with a dedicated reagent system enabling BACT-specific staining; thus, a very sensitive BACT detection is possible with this instrument. BACT concentrations down to levels between 10^5 and 10^6 BACT/L can be determined by the instrument (15-17). Consequently, BACT quantification performed by using UF-100 and UF-1000i analyzers showed a less satisfactory correlation than for data observed for WBC. By using the Bland-Altman plot we observed an overestimation of BACT quantification by UF-100 vs. UF-1000i. It should be pointed out that the experimental cut-off value obtained in this study for Sysmex UF-1000i was $1.25x10^8$ BACT/L, quite similar to the cut-off value adopted for cultural examination.

We also performed a comparison concerning the concordance in sample classification between UF-100 and UF-1000i. As reported in Table 2, despite of the problems in BACT quantification showed by UF-100, the sample classification in comparison with quantitative culture was satisfactory for both UF-100 and UF-1000i systems. These results underline a satisfactory performance of both Sysmex UF-100 and UF-1000i analyzers in rapid diagnosis of UTI, with a better specificity for UF-1000i due to an improved method for BACT quantification. By using UF-1000i and considering only BACT quantification, we observed a SE of 0.97 and a SP of 0.94 when results were compared to cultural examination. If this analyzer is used as a screening method to discharge negative urine samples, a NPV of 98% in comparison with the standard culture method is obtained.

REFERENCES

1. Kass EH. Asymptomatic infections of the urinary tract. Trans Assoc Am Physicians 1956;69:56-64.
2. Kass EH. Pyelonephritis and bacteriuria. A major problem in preventive medicine. Ann Intern Med 1962;56:46-53.
3. Sanford JP. Urinary tract infections. Ann Rev Med 1975;26:485-9.
4. Manoni F, Fornasiero L, Ercolin M, et al. Cutoff values for bacteria and leukocytes for urine flow cytometer Sysmex UF-1000i in urinary tract infections. Diagn Microbiol Infect Dis 2009;65:103-7.
5. Manoni F, Valverde S, Antico F, et al. Field evaluation of a second generation cytometer UF-100 in diagnosis of acute urinary tract infections in adult patients. Clin Microb Infect 2002;8:662-8.
6. Evans R, Davidson M, Sim L, et al. Testing by Sysmex UF-100 flow cytometer and with bacterial culture in a diagnostic laboratory: a comparison. J Clin Pathol 2006; 59:661-2.
7. Koken T, Aktepe O, Sertesr M, et al. Determination of cut-off values for leukocytes and bacteria for urine flow cytometer (UF-100) in urinary tract infection. Int Urol Nephrol 2002;34:175-8.
8. Kim S, Kim Y, Lee S, et al. Evaluation of the Sysmex UF-100 urine cell analyzer as a screening test to reduce the need for urine cultures for community acquired urinary tract infection. Am J Clin Pathol 2007;126:922-5.
9. Regeniter A, Haenni V, Risch L, et al. Urine analysis performed by flow citometry: reference range determination and comparison to morphological findings, dipstick chemistry and bacterial culture. Results of a multicentric study. Clin Nephrol 2001;55:384-92.
10. Hannemann-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.
11. Kouri TT, Kahkonen U, Malminiemi K, et al. Evaluation of Sysmex UF-100 urine flow cytometer vs chamber counting of supervitally stained specimens and conventional bacterial cultures. Am J Clin Pathol 1999;112:25-35.
12. Zamann Z, Roggeman S, Veerhaegen J. Unsatisfactory performance of flow cytometer UF-100 and urine strips in predicting outcome of urine cultures. J Clin Microbiol 2001;39:4169-71.
13. Wang J, Zhang Y, Xu D, et al. Evaluation of Sysmex UF-1000i for urinary tract infection diagnosis. Am J Clin Pathol 2010;133:577-82.
14. vonWulffen H. Evaluation of a new automated urine cell analyzer (Sysmex UF-1000i) for bacteriological urinalysis. XVII European Congress od the European Society of Clìnical Microbiology and Infectious Diseases. Munich April 2007, Abstract n°1733.
15. Terajima S, Yokomizo H, Yagi A, et al. Evaluation study for reference intervals of urine sediments using UF-1000i in medical checkup population. Sysmex Journal International 2009;19:82-6.
16. Terachi M, Nishida Y, Funahashi J, et al. Evaluation study for reference interval of UF-1000i using urine specimens from school children. Sysmex Journal International 2009;19:1-4.
17. Manoni F, Fornasiero L, Ercolin M, et al. Quantificazione della batteriuria e dei leucociti urinari mediante Sysmex UF-1000i: confronto con la coltura quantitativa. RIMeL 2009;5:44-9.