Clinica Chimica Acta 484 (2018) 171–178

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Brief reports

Evaluation of the new Sysmex UF-5000 fluorescence flow cytometry analyser for ruling out bacterial urinary tract infection and for prediction of Gram negative bacteria in urine cultures

Rita De Rosa*, Shamanta Grosso, Giada Lorenzi, Graziano Bruschetta, Alessandro Camporese

Microbiology and Virology Department, Pordenone Hub Hospital, AAS 5 "Friuli Occidentale", Via Montereale 24, 33170 Pordenone, Italy

ARTICLE INFO

Keywords:
Bacteriuria screening
Flow cytometer
UF-5000
Urinalysis
Urinary tract infection (UTI)
Urine culture

ABSTRACT

Background: We evaluated the new flow cytometer UF-5000 with a blue semiconductant laser as a screening tool for ruling out urine samples negative for UTI and its ability to predict Gram negatives in culture.

Methods: Flow cytometry and microbiological analysis were performed on 2719 urine samples, sent to our microbiology laboratory with a request for urine culture.

Results: UF-5000 showed a very good performance in the screening process. Carryover and cross-contamination was negligible. 797 samples were culture positive at a cut-off of $\geq 10^5$CFU/mL. ROC curve analysis for BACT count demonstrated AUC between 0.973, on 2714 samples, 0.959, on 1516 female samples, and 0.988 on 1198 male samples, respectively. At the cut-off of BACT ≥58/μL AND/OR YLC ≥150/μL, SE was 99.4%, SP 78.2%, PPV 65.4% and NPV 99.7%; false negatives were 0.6%, avoiding unnecessary cultures in 55.5% of specimens. "Gram Neg?" flag predicted Gram negatives in culture with a SE of 81.6% and SP of 93.3%.

Conclusion: The new Sysmex UF-5000 showed high diagnostic accuracy in UTI-screening with a very low rate of false negatives. The instrument is capable of predicting Gram negatives with a good SE and a high agreement with the culture, even if this performance needs further evaluation.

1. Introduction

It is well known that urinary tract infections (UTIs) are among the most frequent infections in both hospitalized and outpatients. As a result, urine specimens constitute a significant proportion of routine microbiology laboratory workload. A diverse spectrum of pathogens, Gram negatives, Gram positives and yeasts, with high predominance of members of the family *Enterobacteriaceae*, in particular *Escherichia coli*, are responsible for these infections [1]. Therefore their antibiotic treatment should be as targeted as possible to ensure optimal treatment, prevention of resistances and, last but not least, cost efficacy [2]. Traditional culture remains the "gold standard" for the diagnostic evaluation of patients suspected of having a UTI: it allows to identify the etiological agents, to estimate the concentration of isolated microorganisms and to offer susceptibility testing for targeting the optimal antibiotic therapy [3]. However this method is laborious, time-consuming and expensive. Moreover, up to 80% of urine samples submitted to the laboratory turn out to be negative.

As a prompt laboratory diagnosis is not available, clinicians usually initiate empirical antibiotic treatments without supportive laboratory evidence, which leades to antibiotics over-prescription and increased risks for resistencies.

During the last 20 years fully automated instruments for particle urinalysis, including bacteria, leucocyte, yeast, erythrocyte and epithelial cell counting have been developed to rule out negative samples before processing them in culture with high efficiency in specimens handling, thus avoiding unnecessary culture tests and saving costs for patients and laboratories. Rapid screening for UTI also helps to reduce the turnaround time (TAT) and negative results can be reported at the day of sample collection [4–7]. Furthermore, the clinical decision could take advantage from the rapid prediction of the type of microorganism before the culture results are available, thus allowing to go for a more specific antibiotic treatment.

Many studies and a recent meta-analysis showed that the

Abbreviations: AUC, area under the curve; BACT, bacteria; B_FSC, bacteria forward scatter; B_FLH, bacteria fluorescent light intensity; Ch, analytical channel; CFU, colony-forming unit; CI, confidence interval; CHRO, CHROMagar™ Orientation; CNA, colistin-nalidixic acid; LIS, laboratory information system; NPV, negative predictive value; PPV, positive predictive value; RBC, red blood cell; ROC, Receiver Operating Characteristic; SE, sensitivity; SP, specificity; SFL, side fluorescence light; SSC, side scatter light; TAT, turn around time; UTI, urinary tract infection; WBC, white blood cell; YLC, yeast-like cell

* Corresponding author.
E-mail address: rita.derosa@aas5.sanita.fvg.it (R. De Rosa).

https://doi.org/10.1016/j.cca.2018.05.047
Received 15 March 2018; Received in revised form 18 May 2018; Accepted 23 May 2018
Available online 24 May 2018

parameters bacteria and WBCs could be detected with high sensitivity (SE) by automated urine sediment analyzers and that both are good measures for UTI screening [8]. Among these analyzers, flow cytometers of the UF-Series are widespread in many clinical microbiology laboratories as they have proved performances in line with the EUG guidelines. With the UF-1000i, the second generation of UF-analysers by Sysmex, the analytical quality has been improved due to a dedicated analytical channel for bacteria [9–16].

Having this in mind, we aimed to verify the performance of the new UF-5000 fluorescence flow cytometry analyser as a method to screen out urinary tract infection, also evaluating the ability of the newly introduced Bact Info flag to differentiate Gram negative bacteria in UTI-samples.

2. Materials and methods

2.1. Study protocol and samples

This study was undertaken to evaluate the analytical performance and the diagnostic accuracy to screen out for UTI of the new fully automated UF-5000 fluorescence flow cytometry analyser, in comparison with quantitative standard culture, and to verify the instrumental ability of the new Bact Info flag to differentiate Gram negative bacteria in UTI suspected samples.

The study, conducted according to the Declaration of Helsinki and deemed exempt from the Regional Friuli-Venezia Giulia Ethics Committee was executed from 06 April to 12 June 2017.

During this period 2719 midstream urine samples were evaluated from 1198 (44.1%) male and 1521 (55.9%) female, aged from 0 and 97 years (median = 66y), submitted to our microbiology laboratory with a specific request for urine culture. Inpatients were 23.9% and outpatients 76.1%. Written and/or oral instructions were provided to collect the specimens in a sterile leakproof container (60 mL) fully equipped for sampling by preservative-free vacuum PET tubes (Vacutest Kima, Arzergrande PD, Italy). Immediately after sample collection, the tubes were filled through the straw of the container and, if analysis was delayed by > 1 h, stored and transported at controlled temperature of 4–8 °C to the laboratory.

Specimens were in the first line excluded from the study because the available sample volume was of < 5 mL or the sample was positive for exclusion criteria as indicated by the manufacturer (abundant mucus, high turbidity, macroscopic pyuria and hematuria) to prevent both instrumental failures and interferences during the measurement. All specimens passing the criteria were analyzed with the Sysmex UF-5000 in accordance with the manufacturer's recommendations immediately after inoculation of the culture at the same day of collection and within one hour after the arrival in the laboratory. None of the specimens analyzed were excluded from evaluation.

Gram staining of centrifuged specimens was conducted in case of discrepancies between the results of the UF-5000 Bact Info flag and the culture method. The samples were stored at 4–8 °C until culture analysis and Gram staining procedure was performed and both results were available.

2.2. Sysmex UF-5000

The UF-5000, the third generation of fully automated flowcytometry analysers for the particle analysis in urine, has recently been lauched by Sysmex Corporation (Kobe, Japan). The analyser can discriminate and count 17 diagnostic parameters of cells and formed elements in urine and offers an integrated body fluid mode (BF), available on the instrument with a switch, that can classify and count seven diagnostic parameters. The system employs fluorescence flow cytometry technology, using a new blue semi-conductor laser at 488 nm wavelength, hydrodynamic focusing in two different analysis chambers, surface (SFch) and core (CRch). Particles are stained by specific fluorochromes for nucleic acids and for surface structures, then sent through the laser beam. Counting and classification is based on signals of forward scattered light (FSC), side scattered light (SSC), side fluorescent light (SFL) and the new, additional depolarized side scattered light (DSS). The pattern of individual light signals is translated by specific algorithms into individual 'fingerprints' allowing counting, identification and classification into the particle categories.

Compared to the previous cytometers of the UF-Series, technological innovations aimed to improve the SE and the specificity (SP) for some elements of urinary sediment, particularly for the determination of bacteria. Further on the performance for yeast detection seems to be better in UF-5000 [17]. The light signals of FSC, SFL and SSH differ for Gram negatives and Gram positives due to a different dye intake by the cell wall structures. On this basis UF-5000 provides information on the Gram morphology of bacteria displayed as the Bact Info flag. The "Gram Neg?" flag can be considered particularly interesting for rapid identification of Gram negative microorganisms causative for UTI.

The Sysmex UF-5000 has a maximum theoretical throughput declared of 105 samples/h, requiring a minimum volume of 2.0 mL of uncentrifuged, native urine sample in an automated mode, or 0.6 mL in a STAT mode available for both urine and BF mode. In automatic and STAT mode, the aspiration volume is 0.45 mL for both urine and BF.

2.3. Microbiological analysis

On all the samples, a standard quantitative urine culture was performed inoculating 1-μL of well-mixed urine specimen by a calibrated loop both on a nonselective chromogenic agar plate (CHRO CHROMagar Orientation, Kima Arzergrande, PD, Italy) supporting the growth of UTI pathogens and on a selective colistin-nalidixic acid agar plate with 5% sheep blood (CNA Columbia agar, Kima Arzergrande, PD, Italy). CHRO was used as a quantitative reference; CNA enables isolation and preliminary identification of Gram positive bacteria and allows to discriminate contaminants from uropathogenic species easier. The plates were incubated aerobically at 35–37 °C for 18–24 h and examined for significant bacteriuria. The results were expressed as the number of colony forming unit per milliliter (CFU/mL). For the purposes of this study, cultures that presented microorganisms usually causative of UTI with growth of 10^5 CFU/mL or more were considered positive and these microorganisms were identified by using an automated instrument (Vitek 2; bioMérieux) or by conventional biochemical methods which are used in our laboratory. If more than two organisms were grown in culture, although UTI is unlikely in these patients, the specimen was considered positive and reported as "mixed flora". In these samples bacteria were not subjected to the identification procedure. A specimen was considered negative if there was no growth or there was $< 10^5$/mL bacterial growth, interpreted as insignificant growth.

2.4. Carryover analysis

Rinsing steps between samples were used in all analyses. In this mode carryover evaluation for bacteria and WBCs was performed by measuring specimens with high values of the two parameters (BACT mean value = 99,359/μL; WBC mean value = 1805/μL) in triplicate, followed by a triplicate of specimens with very low values (blank). This serie was consecutively analyzed three times. The carryover was determined by the formula: Carryover = (blank 1–blank 3)/(high 3–blank 3) for all three runs and mean values were calculated for each parameter. Cross-contamination, ***i.e.*** transfer of cells or particles from one sample tube to the following one, was also evaluated. Four racks were prepared, each one containing a positive sample with high bacteria count $\geq 10^6$ CFU/mL followed by three aliquots of a low bacteria count (negative) sample.

After the racks were run on the analyser, all the tubes were cultured in order to observe if there was a cross-contamination in the negative

samples.

2.5. Data analysis

The results of the Sysmex UF-5000 BACT and WBC counts were compared with the results of urine cultures using Receiver Operating Characteristic (ROC) curve analysis. SE, SP, positive (PPV) and negative predictive value (NPV) were calculated for the two parameters at different cut-offs with respect to the reference urine culture test at a limit of 10^5 CFU/mL. Diagnostic accuracy of bacterial and WBC counts for UTIs was assessed by the area under the ROC curve (AUC). The Youden index [calculated as the maximum = (SE + SP-1) value] method was used to estimate the best cut-offs to discriminate positive and negative samples. Statistical analysis was performed with the software Analyse-it for Microsoft Excel (Analyse-it Software, Leeds, United Kingdom).

3. Results

The evaluation could not identify any carryover or cross-contamination. With the protocol described here, the carryover rates of WBC and BACT count were 0.00016 and 0.00003, respectively. Furthermore, from the microbiological point of view, cross-contamination could be excluded because none of the cultured negative samples exhibited a higher growth in culture than one $\log_{10}$ over the bacterial count when run on the UF-5000 at the end of a series of positive samples.

Of the 2719 samples, 1922 (70.7%) were negative in urine culture (sterile or $\leq 10^4$ CFU/mL) and 797 (29.3%) showed significant growth. In 574 of the positive samples a single microorganism was identified, in 71 samples two microorganisms were isolated, and 152 samples showed mixed flora. Although UTI is unlikely in these patients, these samples were considered gold standard positive when the growth of bacteria exceeded the gold standard definitions of a negative culture, *i.e.* 10^5 CFU/mL. The microorganisms isolated reflected the usual rate of uropathogens in our laboratory, so that the most frequent bacteria reported were Gram negatives accounting for 76.9% of all species isolated, in the majority *Enterobacteriaceae*. Five samples showed growth for yeasts (*Candida spp*) (Table 1).

Excluding the samples culture positive for yeasts, ROC curve analysis on the BACT count results was performed with the total of 2714 specimens, including 1516 specimens collected from females and 1198 specimens collected from males. Considering all the samples in total, AUC was 0.973 (C.I. 0.968 to 0.977; SE 0.0025) (Fig. 1). ROC curve for the female and male samples separated showed an AUC of 0.959 (C.I. 0.950 to 0.967; SE 0.0043) and 0.988 (C.I. 0.984 to 0.993; SE 0.0024), respectively (Fig. 2). The performance of UF-5000 at different cut-offs for the BACT count was investigated for all specimens in total, as well for the cohorts of males and females separately. Taking into account the clinical SE as well as the efficiency of the screening process, the best cut-off seemed to be 58 BACT/μL.

Table 1
Microorganisms identified in the 797 culture positive samples.

Microorganism identified	n	%
E. coli	414	57.9
E. faecalis	83	11.7
K. pneumoniae	54	7.6
Streptococcus/Aerococcus spp	25	3.5
P. mirabilis	21	2.9
S. agalactiae	20	2.8
P. aeruginosa	16	2.2
C. koseri	11	1.5
M. morganii	11	1.5
K. oxytoca	8	1.1
Other Gram positives	32	4.5
Other Gram negatives	16	2.2
Candida spp.	5	0.7

At this cut-off we found five false negatives in culture: three samples showed growth of mixed flora (two samples at 10^5 CFU/mL and one sample at $5*10^5$ CFU/mL). The other two samples were positive exhibiting 10^5 CFU/mL of *Escherichia coli* with 13 BACT counts/μL for a female sample, and *Enterococcus faecalis*, with 34 BACT counts/μL for a male sample. Investigating the male and female groups separately, the best cut-off for males seemed to be 25 BACT/μL and for the female 58 BACT/μL. At these gender cut-offs, false negatives were reduced to three: two male samples showing in culture mixed flora, and one female sample showing *Escherichia coli* at 10^5CFU/mL. The results are summarized in Table 2.

ROC curve analysis was also performed for WBC count *versus* urine culture on the total of 2719 samples, and on the 1198 male and 1521 female, since this parameter could contribute to the performance with regard to UTI screening. For all the samples AUC was found to be 0.844 (C.I. 0.827 to 0.60; SE 0.0083), for the female samples it was 0.816 (C.I. 0.794 to 0.838; SE 0.0112), and for the male samples 0.873 (C.I. 0.845 to 0.901; SE 0.0142), respectively (Fig. 3). In Table 3 the diagnostic performance is shown at a cut-off of 40 WBC/μL. In our evaluation WBC count could not improve the diagnostic accuracy of the screening, not even in combination with BACT count and YLC count (Table 4). Indeed, for example, when we combined the WBC count at a cut-off of 40/μL, with the cut-off of ≥150/μL for YLC and the best cut-off for BACT count (58/μL), the false negative samples were reduced from five to three, but the false positives increased from 419 at 547.

Regarding YLC count, the median count of the analyser was 13/μL in culture negative samples and 377/μL in culture positive samples for yeasts. The optimal cut-off that allowed the maximum SE and NPV with acceptable SP was selected experimentally at 150 YLC/μL obtaining SE 100%, SP 98.3%, NPV 100% and agreement of 98.3% respect of culture results. Out of the total of 2719 samples evaluated, 51 samples with YLC counts above this cut-off were identified as positive (1.9%). Compared to culture, five samples of 51 were classified correctly as positive for *Candida spp* and 46 resulted as false positive. Among these 46 samples, 43 (93.5%) showed BACT counts above the cut-off of 58/μL, of which 29 were culture positive for bacteria and 14 were culture negative. 35 samples, including 11 of the 14 culture-negative samples, highlighted high RBC counts; in 17 of these cases the device showed the flag “RBC/YLC misclassification” helping to identify false positive samples for YLC. Furthermore, two culture negative female samples showed high squamous epithelial cells counts suggesting a contaminated urine collection. Only three samples were false positive for YLC count exclusively. The instrumental performance was also evaluated combining BACT count with YLC count ≥150/μL (*i.e.* when BACT AND YLC count as well as BACT OR YLC count were above the defined cut-off) for the cohort of all samples. The best results were achieved with BACT ≥58/μL AND YLC ≥150/μL. These results are summarized in Table 4.

Within the 2719 samples, the new Bact Info-flag “Gram Neg?” was activated in 493 of 1211 samples with bacteria count > 58/μL, considered positives at the screening process in this study. Among the samples flagged, 475 (96.3%) were culture positive and 18 (3.6%) culture negative. Except for one case, all the samples where “Gram Neg?” was indicated, exhibited at least one Gram negative bacteria species in culture and/or microscopic evaluation of Gram staining. In total we found a full agreement between Gram classification of the flag “Gram Neg?” in 411 samples (86.5%), a partial agreement (namely when Gram positives and Gram negatives were identified), in 63 samples (13.3%) and one sample disagreed (0.2%) as shown in Table 5. The SE of this parameter was 81.6%, the SP was 95.9%, the PPV 95.8% and the NPV 81.7%. Over the 1922 negative culture samples, “Gram Neg?” flag started in 18 samples (0.9%), however in these samples, Gram negatives were detected in 15 samples (83.3%) to a non-significant degree in culture or by microscopy.

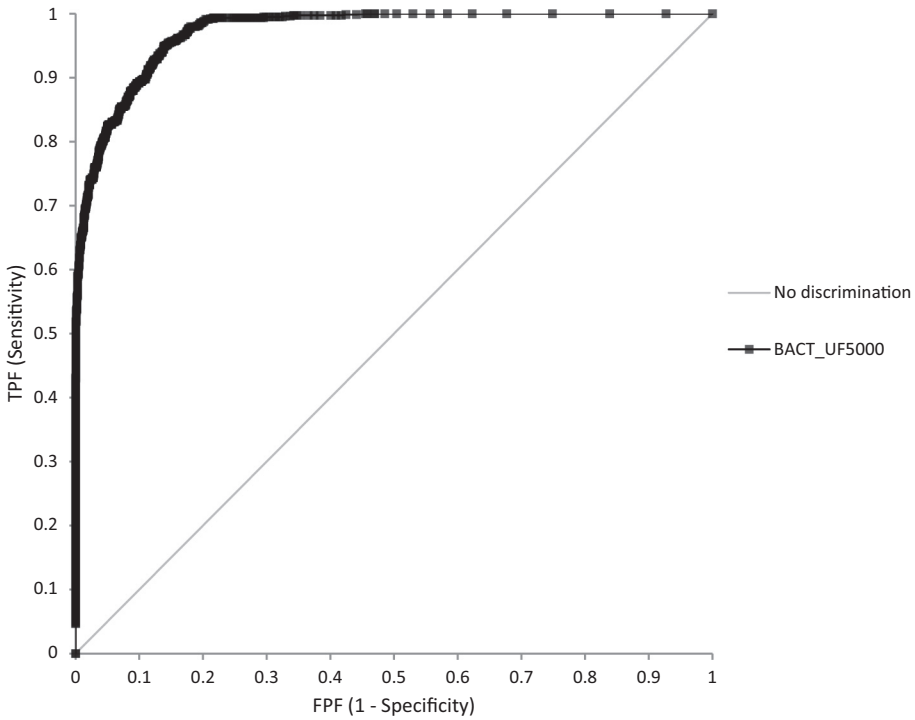

Fig. 1. ROC curve for UF-5000 bacterial count (BACT_UF5000) *versus* urine quantitative culture in 2714 samples (792 cases positives for bacteria in culture). Urine cultures were considered positive if the bacterial growth was $\geq 10^5$ CFU/mL.

4. Discussion

In a recent evaluation UF-5000 was validated for detection and enumeration of 17 urinary parameters related to the pathological process of kidney and urinary tract [17]. For the first time, to our knowledge, we evaluated the new generation of Sysmex UF-5000 fluorescence flow cytometry analyser as a method to rule out negative urine samples for UTI, in comparison of urine culture. Performances analysis showed that UF-5000 was suitable for UTI screening. Carryover and cross-contamination were not significant and this is an important concern in the microbiology screening, as the same tube could be employed for urine seeding when the screening result is positive.

Urine culture is still the gold standard method for laboratory diagnosis of UTI but has drawbacks in the laboratory workflow, and above

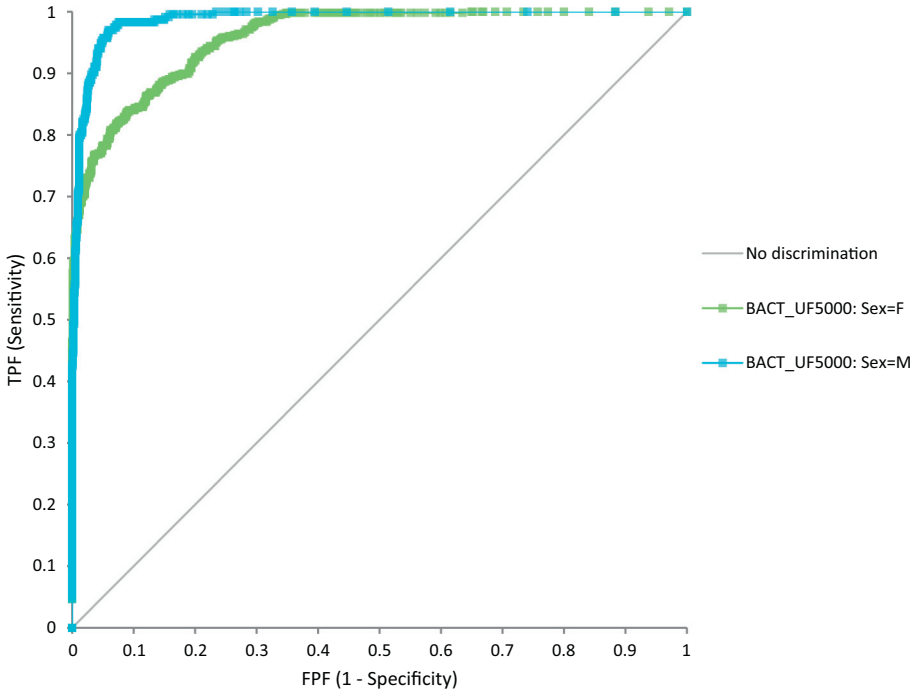

Fig. 2. ROC curve for UF-5000 bacterial count (BACT_UF5000) *versus* urine quantitative culture in 1516 samples from female and 1198 samples from male (557 positive samples from female and 235 positive samples from male). Urine cultures were considered positive if the bacterial growth was $\geq 10^5$ CFU/mL.

Table 2
Diagnostic performance of UF-5000 at different cut-off values for BACT count in comparison of quantitative urine culture evaluated in 2714 samples without 5 samples positives for yeast in culture.

Cut-off Bact count (cells/μL)	Sensitivity %	Specificity %	Negative Predictive value (NPV) %	Positive Predictive value (PPV) %	True Positives n	False positives n	True negatives n	False negatives n
Total								
33	99.5	70.4	99.7	58.1	788	568	1354	4
58	99.4	78.4	99.7	65.4	787	416	1506	5
64	99.1	79.6	99.5	66.6	785	393	1529	7
71	98.5	80.4	99.2	67.4	780	377	1545	12
Males								
20	99.6	81.1	99.99	68.0	234	182	781	1
25	99.1	84.8	99.99	73.0	233	146	817	2
58	98.3	91.6	99.0	83.0	231	81	882	4
Females								
58	99.8	65.2	99.99	54.0	556	334	625	1
63	99.5	66.8	99.99	55.0	554	318	641	3

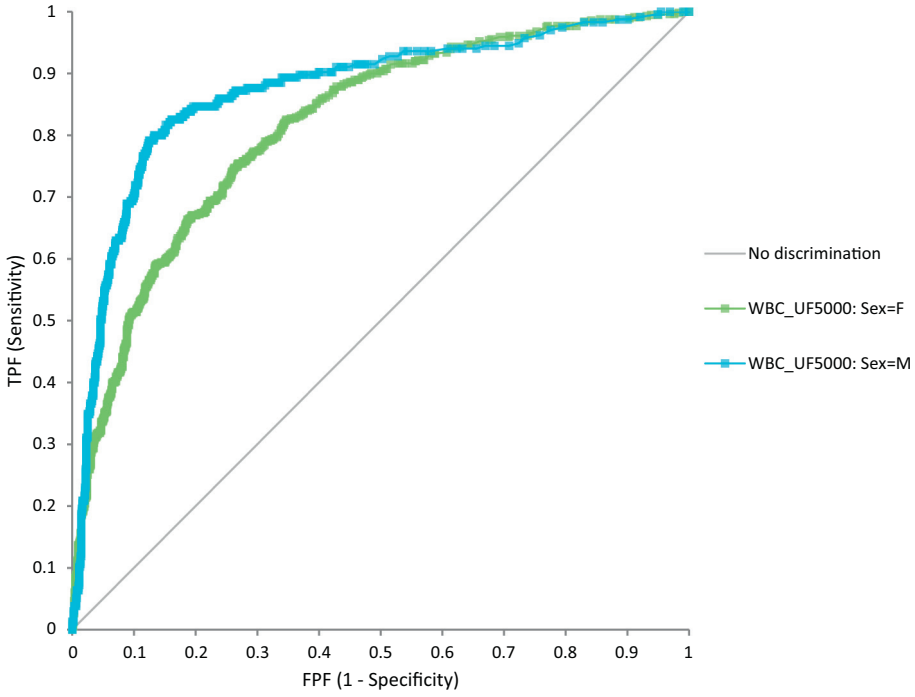

Fig. 3. ROC curve for UF-5000 leucocytes count (WBC_UF5000) *versus* urine quantitative culture in 1521 samples from female and 1198 samples from male (552 positive samples from female and 235 positive samples from male). Urine cultures were considered positive if the bacterial growth was $\geq 10^5$ CFU/mL.

Table 3
Diagnostic performance of UF-5000 at a cut-off of 40 WBC count/μL in comparison of quantitative urine culture evaluated on the total of samples and for gender.

Specimens n (Sex)	Sensitivity %	Specificity %	Negative Predictive value (NPV) %	Positive Predictive value (PPV) %	True positives n	False positives n	True negatives n	False negatives n
2719 (F + M)	70.9	85.3	87.6	66.6	539	282	1640	258
1521 (F)	63.0	83.0	84.0	61.0	354	163	796	208
1198 (M)	78.7	87.6	91.0	73.0	185	119	844	50

all, its clinical efficacy is limited due to high TAT. First results after 18-24 h can neither help to reduce unnecessary prescription of antibiotics, nor to predict the infection in an acceptable time. Many studies have demonstrated that the flow cytometers of the first, and even more of the second generation (UF-1000i), seem to meet the needs of a rapid screening method in order to reduce the number of cultured samples that exhibit a non-significant or sterile growth. These works had shown that counts of bacteria particles are the optimal indicator for positive culture results [8–13].

Our results demonstrate that BACT count at a cut-off of 58/μL, in combination with the experimental YLC count $\geq$150/μL are the optimal indicator for positive culture results with a high SE (99.4%) as required for a screening method, and are in line with the requirements of the guidelines for microbiological examination of urine. At this cut-off, false negatives were 0.6% of all the positive samples that is an acceptable rate as it is lower than 2% maximum allowable false negative rate for colony concentration $\geq 10^5$ CFU/mL [3]. Between these samples, three were culture positive for mixed flora with absence of

Table 4
Diagnostic performance of UF-5000 at different cut-off values for BACT count AND/OR YLC ≥ 150/μL in comparison of quantitative urine culture evaluated in all the 2719 samples.

BACT ≥ (cells/μL)	WBC ≥ (cells/μL)	YLC ≥ (cells/μL)	Sensitivity %	Specificity %	Negative predictive value (NPV) %	Positive predictive value (PPV) %	True positives n	False positives n	True negatives n	False negatives n
58	–	150	99.4	78.2	99.7	65.4	792	419	1.503	5
64			99.2	79.4	99.6	66.6	791	396	1.526	6
71			98.6	80.2	99.3	67.3	786	381	1.541	11
58	40	150	99.6	71.5	99.8	59.2	794	547	1375	3
64			99.5	72.3	99.7	59.8	793	533	1389	4
71			99.1	72.9	99.5	60.3	790	521	1401	7

Table 5
Results of culture of the samples flagged with "Gram NEG?".

Bact Info flag from urine culture identification or by Gram stain	UF-5000 Bact Info flag gram NEG?
Gram negatives	411
Gram positives + Gram negatives	24
Mixed flora (All the samples showed presence of Gram negatives)	39
Gram positives	1
Yeasts	0
Culture negative (no growth or < $10^{\wedge 5}$CFU/mL)	18
Total	493

leucocyturia suggesting a low possibility of UTI and a higher likelihood for colonization or contamination. Two samples were positive for uropathogens, at a load of 10^5 CFU/mL, and in one leucocyturia was absent. Anyway, the screening strategy could be slightly improved when the screening cut-offs for UF-5000 were chosen separately for men and women. In fact, as it is shown by the ROC curve analysis, the diagnostic accuracy was slightly better for males than for females (AUC = 0.988 *versus* AUC = 0.959). For women this is probably due to contamination by bacteria, WBCs and squamous epithelial cells, during the sample collection procedure for anatomical issues. Applying these gender thresholds, the SE remained 99.4%, with only one false negative for *Escherichia coli* at a concentration of 10^5 CFU/mL which exhibited a mild leucocyturia. At this point the false positives increased by 2.4%. For a screening strategy, UF-5000 showed an optimal SE for detecting positive samples with a cut-off in culture of $\geq 10^5$ CFU/mL and an accepted percentage of false positives of 15.3%, thus avoiding unnecessary urine cultures in 55.5% of all specimens.

As we have experience with the UF-1000i that has been used in our laboratory since 2009 for UTI screening, we compared the diagnostic performances of the UF-5000 with that of UF-1000i (data not shown). For the total of all samples, AUC slightly increased for the UF-5000 (0.971) compared to UF-1000i (0.967) with statistically significant difference (p-value = .0208). Comparing the performances of the two instruments for BACT count by gender, it was shown that UF-5000 exhibited an AUC of 0.956 for females and 0.988 for males, while AUC of UF-1000i was 0.947 and 0.990, respectively.

For the UF-1000i similar results are published in the literature, although at different cut-offs. Giesen et al., using a cut-off of 288.9 BACT/μL and 31.8 WBC/μL assessed SE 93% and SP 86% for a cut-off in culture > 10^5 CFU/mL, with an AUC for bacteria of 0.95 and for WBCs of 0.86 [14]. Manoni et al., found SE 99%, SP 77%, and NPV 98% by using culture positivity criteria $\geq 10^*10^5$ CFU/mL, at bacteria cut-off over 125/μL and/or WBC over 40/μL [9].

In a study performed with the UF-500i on 1094 samples, AUC for bacteria and WBCs were 0.944 and 0.902, respectively. The authors used different sets of cut-off values for all the samples as well as age- and gender-specific cut-offs. For the combined optimal cut-off values, 630 BACT/μL and 10 WBC/μL for women and 40 BACT/μL and 17 WBCs/μL for male and children, SE and SP were 97% and 77.7%, respectively, leading to a reduction of urine cultures of 64.5% with a false positive rate of 3% [12].

The diagnostic performance in terms of SE and SP differed among the published studies due to different cut-off values for BACT and/or WBC count, but also due to different criteria for the diagnosis of UTI, different prevalence of the disease and characteristics of the studied population. For these reasons it is appropriate that each laboratory establishes its' optimal cut-offs based on the characteristics of the prevalent population and the different positivity-criteria for the culture, moreover these limits should be periodically reviewed and verified.

Interpretation of screening efficiency is complex and the analyser performance depends on many issues. However, in many evaluations, automated flow cytometers have shown better diagnostic performances than instruments based on other measurement principles.

For example, evaluating 308 samples from both inpatients and outpatients, the work of Gras et al. showed that FUS-200 - a urine sediment analyser that combines flow cytometry with digital imaging identification - pointed out AUC for bacteria count of 0.82 (95% CI 0.78–0.86) and for WBCs 0.81 (95% CI 0.76–0.85), at a cut-off of > 10^5 CFU/mL for culture. With unedited results, the best FUS-200 cut-off value for detecting bacteriuria was 4.4 *10^6 bacteria/L, with a SE of 79% and SP of 67.6%, and that for WBCs was 12.3 × 10^6 WBC/L, with SE of 65% and SP of 75.9%. The same study founded that the SediMAX - the automated analyser based on the visual microscopy analysis of centrifuged urines- showed AUCs for the bacterial and WBC counts of 0.73 and 0.77, respectively. At a bacteria cut-off of 75 × 10^6 cells/L, SE was 65% and SP was 63%, while at WBC > 48.4 × 10^6 cells/L, SE was 47.5% with a SP of 96.3% [18].

In a more recent evaluation of sediMAX, AUC values for both leukocyte (0.687; 95% CI: 0.665–0.729) and bacterial count (0.587; 95% CI: 0.551–0.623) showed that WBCs were a better predictor for positive culture results than bacterial cell count. However, neither parameter generated SE or SP within acceptable limits for a diagnostically useful test (AUC ≥ 0.9) [19].

In the paper of Stürenburg et al. evaluating the iQ200, which analyzes a urinary sample using flow imaging technology with auto particle recognition, the "all small particles (ASP)" count were the only individual indicator that developed 95% SE, with a SP of 44.2% and saving 25.8% of samples to be cultured. When the regression model was used to determine possible cut-off values for a combination of parameters (leukocytes AND bacteria, AND "ASP"), at a cutoff value of > 10^4 CFU/mL for a positive culture, a fixed SE of 95% resulted in a SP of 61% and a reduction in urine cultures of 35% [20].

We found an AUC value for the UF-5000 for WBC of 0.844, slightly higher than for UF-1000i (0.833) with a statistically significant difference (p-value < 0,0001). UF-5000 showed a very good correlation with UF-1000i for WBC count in the total of samples examined (r = 0.979; C.I. = 0.977 to 0.981). On the contrary, the BACT count correlation was of a lower degree (r = 0.628; C.I. = 0.6050 to 0.6505). The hardware changes and new reagents for the particle classification could explain the difference between UF-5000 BACT count in comparison with UF-1000i, therefore the correlation might be smaller. This could also

explain the different optimal cut-offs for UTI-screening between the two instruments, even because the new reagents in different modes could affect the tendency of the bacteria to aggregate.

As said previously, in the majority of the studies, WBC count at defined cut-off values was used alternatively or in combination of BACT count for the best screening process. [9–11]

Even if we consider WBC count an essential parameter for clinical evaluation of culture results, as it helps to estimate asymptomatic bacteriuria or contamination from infection, in this evaluation of UF-5000, WBC count could not improve the diagnostic accuracy for UTI. In fact, at a cut-off value of 40 WBC/μL, the false negatives were only reduced from five to three in absolute and, on the other hand, the false positive samples increased from 15.2% to 20.3%.

The number of positives for yeast was too low for a statistical evaluation of the YLC count parameter by ROC curve in this study. However the use of the experimental cut-off of 150 YLC/μL allowed to culture all the positive samples for yeasts.

The study has some limitations like the criterion for culture positivity of 10^5 CFU/mL. This was determined on the basis of heterogeneity of the population covered by our laboratory, as well as due to the lack of information on the clinical symptoms and antibiotic therapy. However, if the laboratory had more information on the ongoing antimicrobial treatment, it could evaluate the high number of negative cultures more carefully compared to the instrumental results, as these negative results are caused very often by the presence of dead bacteria or by a low bacterial growth due to the inhibition of antibiotic.

Further verification is required for some subgroups in the population, in particular immunocompromised patients, where the cut-off for significant bacteriuria will be lower, and for which the current screening strategy might not be fitting.

The slide centrifuge method with Gram staining has been used in some microbiology laboratories as a screening method for urine prior to culture, but for this purpose, despite a high SE and SP of 90% against the culture, it has poor efficiency as it is very labor-intensive [5]. On the other hand, this test offers information on bacterial morphology, thus providing relevant information to the clinician in terms of antibiotic treatment before the results of urine culture are available. Some studies have already evaluated the capability of a rapid presumptive discrimination of Gram negatives from Gram positives on Sysmex UF-1000i, using two technical parameters reported by the instrument, the bacterial forward scatter (B_FSC) and the bacterial fluorescent light intensity (B_FLH), that provide information on size and content of bacteria particles. In particular B_FSC provides more useful information, as at a cut-off of 30 ch indicated Gram negative bacteria in 97% of cases. [10] A SE of 68% and SP of 84% at a cut-off of 25 ch for presumptive identification of Gram negatives was reported in another recent work [21].

The ability of the new Bact Info flag of UF-5000, “Gram Neg?” demonstrated a good SE and optimal SP to predict Gram negatives in culture. The instrumental interpretative algorithm for the bacterial counts above which to trigger the flag can be set according to the user's choices. In our study it was set at the bacterial cut-off for optimal UTI-screening, in order to provide this information for clinically relevant cases. Under these conditions, the flag showed a high agreement (99.8%) with culture where Gram negatives alone or Gram negatives together with Gram positives were detected, with very low discordance (0.2%).

In conclusion, the UF-5000 was represented as a rapid and reliable method for ruling-out UTI with a similar performance like the UF-1000i, but with a slightly better diagnostic accuracy in women. Moreover, with respect of the previous generation of analyzers it offers the chance to detect the Gram negative bacteria in very high agreement with urine culture. To know the morphology of the UTI-causing bacteria as soon as possible is important, even more in cases of UTI complicated with bacteraemia or sepsis where a targeted therapy could improve the patient's outcome.

Ethical approval

All procedures were in accordance with institutional and national ethical standard.

Authors' contribution

All the authors confirmed they have contributed to the intellectual content of this paper and have met the following requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Research funding

The study was financially supported by Sysmex Europe GmbH.

Authors' conflict of interest disclosure

All authors stated that there are no conflicts of interest regarding the publication of this article.

References

[1] B. Foxman, The epidemiology of urinary tract infection, Nat. Rev. Urol. 7 (2010) 653–660.
[2] L. Abbo, T. Hooton, Antimicrobial stewardship and urinary tract infections, Antibiotics 3 (2) (2014) 174–192. Available from: http://www.mdpi.com/2079-6382/3/2/174/.
[3] H. Hallander, W. Hofmann, W.G. Guder (Eds.), E. European urinalysis guidelines, Scand. J. Clin. Lab. Invest. 60 (Suppl. 231) (2000) 1–96.
[4] M. Fritzenwanker, FMCTF. Modern diagnostic methods for urinary tract infections, Expert Rev. Anti Infect. Ther. 14 (11) (2016) 1047–1063.
[5] M. Pezzlo, Detection of urinary tract infections by rapid methods, Clin. Microbiol. Rev. 1 (3) (1988) 268–280.
[6] P. Mejuto, M. Luengo, J. Díaz-Gigante, Automated flow cytometry: an alternative to urine culture in a routine clinical microbiology laboratory? Int. J. Microbiol. 4 (2017) 2017 (Article ID 8532736):8 pages).
[7] D. Kayalp, K. Dogan, G. Ceylan, M. Senes, D. Yucel, Can routine automated urinalysis reduce culture requests? Clin. Biochem. 46 (13–14) (2013) 1285–1289.
[8] Y. Shang, Q. Wang, J. Zhang, Y. Xu, W. Zhang, Y. Chen, et al., Systematic review and meta-analysis of flow cytometry in urinary tract infection screening, Clin. Chim. Acta 424 (2013) 90–95.
[9] F. Manoni, L. Fornasiero, M. Ercolin, A. Tinello, M. Ferrian, P. Hoffer, et al., Cutoff values for bacteria and leukocytes for urine flow cytometer Sysmex UF-1000i in urinary tract infections, Diagn. Microbiol. Infect. Dis. 65 (2) (2009) 103–107.
[10] R. De Rosa, S. Grosso, G. Bruschetta, M. Avolio, P. Stano, M.L. Modolo, et al., Evaluation of the Sysmex UF1000i flow cytometer for ruling out bacterial urinary tract infection, Clin. Chim. Acta 411 (1–16) (2010) 1137–1142.
[11] B. Pieretti, P. Brunati, B. Pini, C. Colzani, P. Congedo, M. Rocchi, et al., Diagnosis of bacteriuria and leukocyturia by automated flow cytometry compared with urine culture, J. Clin. Microbiol. 48 (11) (2010) 3990–3996.
[12] S. Jolkkonen, E.L. Paattiniemi, P. Kärpänoja, H. Sarkkinen, Screening of urine samples by flow cytometry reduces the need for culture, J. Clin. Microbiol. 48 (9) (2010) 3117–3121.
[13] K. Kadkhoda, K. Manickam, DeGagne, et al. UF-1000i™ flow cytometry is an effective screening method for urine specimens, Diagn. Microbiol. Infect. Dis. 69 (2) (2011) 130–136.
[14] C.D. Giesen, A.M. Greeno, K.A. Thompson, R. Patel, S.M. Jenkins, J.C. Lieske, Performance of flow cytometry to screen urine for bacteria and white blood cells prior to urine culture, Clin. Biochem. 46 (9) (2013) 810–813.
[15] J. Gutiérrez-Fernández, C. Riazzo, S. Sanbonmatsu, J. de Dios Luna, A. Sorlózano, C. Miranda, et al., Sysmex UF-1000i performance for screening yeasts in urine, APMIS 122 (4) (2014) 324–328.
[16] F. Manoni, A. Tinello, L. Fornasiero, P. Hoffer, V. Temporin, S. Valverde, et al., Urine particle evaluation: a comparison between the UF-1000i and quantitative microscopy, Clin. Chem. Lab. Med. 48 (8) (2010) 1107–1111.
[17] G. Previtali, R. Ravasio, M. Seghezzi, S. Buoro, M.G. Alessio, Performance evaluation of the new fully automated urine particle analyser UF-5000 compared to the reference method of the Fuchs-Rosenthal chamber, Clin. Chim. Acta 472 (July) (2017) 123–130.
[18] J.M. Gras, N. Henry, M. Othmane, G.R. Vranken, Assessment of FUS-200 performance : comparison with quantitative urine culture shows that identifying the right cutoff for optimizing analyzer performance is challenging, J. Appl. Lab. Med. 1 (January) (2017) 365–375.
[19] E. Rosanne Sterry-Blunt, K.S. Randall, M.J. Doughton, S.H. Aliyu, D.A. Enoch, Screening urine samples for the absence of urinary tract infection using the

sediMAX automated microscopy analyser, J. Med. Microbiol. 64 (6) (2015) 605–609.
[20] E. Stürenburg, J. Kramer, G. Schön, G. Cachovan, I. Sobottka, Detection of significant bacteriuria by use of the iQ200 automated urine microscope, J. Clin. Microbiol. 52 (8) (2014) 2855–2860.
[21] G. Gessoni, G. Saccani, S. Valverde, F. Manoni, M. Caputo, Does flow cytometry have a role in preliminary differentiation between urinary tract infections sustained by gram positive and gram negative bacteria? An Italian polycentric study, Clin. Chim. Acta 440 (2015) 152–156.