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ORIGINAL ARTICLE

Comparison between urine culture profile and morphology classification using fluorescence parameters of the Sysmex UF-1000i urine flow cytometer

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Abstract

Aims: To determine the usefulness of the fluorescence parameters generated by Sysmex UF-1000i flow cytometer for the rapid diagnosis of urinary tract infection by bacilli or cocci.
Methods and Results: Urine samples (n = 1924) were studied by culture and microbiology and subsequently by cytometry, using BACT-Morph software and considering forward-scattered light (FSC) and fluorescent light scatter fluorescence parameters. BACT-Morph software showed moderate diagnostic accuracy (78·4%) to detect rod-shaped bacteria, with sensitivity of 82·4% and specificity of 62·5%. Forward-scattered (B_FSC) values of the bacterial channel were significant higher for the Gram-positive cocci category ($P < 0·001$). A cut-off of B_FSC ≥24·2, expressed in arbitrary units (analytical channel, ch), provided higher sensitivity (90·0%) but lower specificity (38·9%), and the diagnostic accuracy for Gram-positive cocci classification reached 62·0%.
Conclusions: Utilization of BACT-Morph software and bacterial channel fluorescence parameters (B_FSC ≥24·2 ch) offered an approximate discrimination of bacilli and cocci but the specificity was low, especially for FSC.
Significance and Impact of the Study: Further research is needed to establish the usefulness of flow cytometry for aetiological diagnosis.

Introduction

Urine tract infections (UTI) are highly frequent in hospitalized patients and in the general community (Foxman 2010). Thus, the incidence of cystitis among women of sexually active age in our region is 18 cases per 10 000. The UTI aetiology varies according to risk factors, previous antibiotic administration, and the hospital or community origin of the UTI. In our setting, *Escherichia coli* is the most frequent pathogen detected in UTIs in both hospital and community samples (Sorlozano *et al.* 2014). The gold standard for UTI diagnosis is urine culture to identify and quantify uropathogens followed by evaluation of their antibiotic susceptibility.

Given the high prevalence of UTIs and large number of samples received by microbiology laboratories, automated screening procedures are needed to rule out nonsignificant bacteriuria (Williams *et al.* 2010) and thereby solely cultivate samples with significant bacteriuria and/or pyuria alone, which can be only 30–40% of urine samples received (Broeren *et al.* 2011; Gutiérrez-Fernández *et al.* 2012; Marschal *et al.* 2012; Muñoz-Algarra *et al.* 2015). The UF-1000i flow cytometer (Sysmex Inc., Kobe, Japan), which quantifies and discriminates bacteria, red blood cells, leucocytes, yeast and other particles, has proven useful to screen urine samples with suspicion of UTI (Gutiérrez-Fernández *et al.* 2012; Marschal *et al.* 2012; Shang *et al.* 2013). The system also has a specific channel for bacteria, improving their identification by avoiding interference from red blood cells, detritus or mucus. The sensitivity and specificity of automated analysers depend on the bacterial cut-off value, which is

selected according to knowledge of the population under investigation. A high sensitivity is essential to minimize false-negative results. Account should be taken of the low capacity of these systems to discriminate contaminated samples or bacteria rendered nonviable by antibiotic treatment (false positives) (Gutiérrez *et al.* 2000).

BACT-Morph software for bacterial morphology identification was recently incorporated into the UF-1000i analyser. In brief, it allows detection of bacterial forward-scattered fluorescence (B_FSC), which denotes particle size, and side-scattered fluorescence, which indicates particle surface and complexity. The nucleic acid contents of each particle can be inferred from observations of the fluorescent light scatter (B_FLH). Bacteria are classified according to their fluorescence emission as rods or cocci/mixed. A scatter plot is constructed of B_FSC against B_FLH values, and the angle defined by a straight line through the data cloud distinguishes between rods (<30°) and cocci/mixed bacteria (>30°) (Geerts *et al.* 2015).

The objective of this study was to determine the usefulness of BACT-Morph software and bacterial channel fluorescence parameters of Sysmex UF-1000i analyser for the rapid discrimination of bacilli and cocci and for the prediction of UTI aetiology.

Materials and methods

Patients and sample collection

During November 2014, a total of 1924 consecutive urine samples were received by the Microbiology Laboratory of Virgen de las Nieves Hospital (University Hospital Complex) in Granada, Southern Spain (428 from males, 1496 from females): 338 were of hospital origin and 1586 of community origin; 30 were from infants, 115 from immunosuppressed adult patients, 112 from catheterized patients, 1068 from adults with no clinical data of interest, and 599 from pregnant women undergoing routine check-up. All individuals were of Caucasian origin. The hospital is a regional reference centre serving a population of around 440 000. Samples were gathered from children using paediatric bags and from adults via urinary catheter or by clean-catch midstream technique, using sterile wide-rim containers or tubes with boric acid (Vacutainer®; Becton Dickinson, Franklin Lakes, NJ). All samples were refrigerated and processed within 24 h of their collection following a previously described procedure (Rojo *et al.* 2011).

Sample assessment

Urine samples were processed following our routine work protocols, ordering culture when Sysmex UF-1000i results showed that one or more of the following criteria were met: ≥40 leucocytes per μl, ≥150 bacteria per μl or ≥100 yeast per μl (Gutiérrez-Fernández *et al.* 2012, 2014). In brief, samples were inoculated on chromogenic agar (CHROMagar Orientation®; Becton Dickinson) with 1 μl loop (Copan, Brescia, Italy) and were then incubated in aerobic atmosphere at 37°C for 18 h. Samples from the Nephrology Unit were additionally inoculated on Columbia blood agar (Becton Dickinson) with 10 μl loop and incubated at 37°C in CO_2 enriched atmosphere for 18 h. Pathogen counts were documented, and the MicroScan® System (Siemens, Barcelona, Spain) was used for micro-organism identification. Fast-growth uropathogens were categorized according to Pezzlo *et al.* (2010) as negative (<10 000 CFU per ml); presumptive (10 000–100 000 CFU per ml of two uropathogens or one uropathogen without leukocyturia); significant (bacteriuria or candiduria with >100 000 CFU per ml of one or two uropathogens or 10 000–100 000 CFU per ml of one uropathogen with leukocyturia); or mixed-contaminated (>10 000 CFU per ml of more than two uropathogens or predominance of urogenital epithelial biota). In yeast-positive cultures, the germ test was conducted in foetal bovine serum to discriminate *Candida albicans* and *Candida* sp. no *albicans*, using the VITEK2® system for species identification (BioMérieux, Madrid, Spain).

In the interpretation of fluorescence data, a single category was assigned when two pathogens with equal morphology were detected. In cases of the growth of two morphologically distinct uropathogens, the morphology of the predominant pathogen was assigned when its growth was >1 000 000 CFU per ml and the growth of the other pathogen was <100 000 CFU per ml. Otherwise, samples were classified as 'mixed cultures'.

Performance using BACT-Morph software and UF-1000i bacterial channel

Subsequent to the culture assessment, urine samples were examined with the UF-1000i flow cytometer following the manufacturer's protocol. Besides providing data on the size of particles, this system uses specific fluorescent dyes (phenanthridine for nucleic acid staining and carbocyanine for membrane staining) for their count and classification. Various bacteria count cut-off values were tested in the evaluation process.

Fluorescence values for the bacterial channel of the system were recorded, expressed in arbitrary fluorescence units. Data were also gathered on the age and sex of patients, the source of the samples and the UF-1000i results for leucocyte (per μl) and CFU (per ml) counts.

Statistical analysis

Descriptive analysis was conducted of sociodemographic data and bacterial fluorescence channel results. Sensitivity, specificity and positive and negative predictive values were assessed for correct rod classification by BACT-Morph software. The Mann–Whitney *U* test was used to evaluate differences in B_FSC and B_FLH values between samples with rods and cocci as identified by culture, because the data distribution was nonnormal. The area under the receiver operating characteristics (ROC) curve (AUC) was calculated to evaluate the correct classification of bacteria morphology based on B_FSC and B_FLH values for the bacterial channel. The effect of contamination and urogenital epithelial microbiota was assessed by comparing results for all samples with those obtained after excluding samples with contamination or microbiota. Finally, the influence on results of the bacteria count in the samples was examined by analysing fluorescence values for samples with different bacteria counts per μl. NCSS10 (Kaysville, UT) was used for all tests, considering $\alpha = 0{\cdot}050$ as significance level.

Ethics statement

The study was carried out in accordance with the Declaration of Helsinki. The study involved no intervention or change to routine work procedures, and biological material was only used for UTI diagnoses ordered by attending physicians, with no additional sampling or modification of the routine sampling protocol. In addition, an anonymous database was used for the analyses. Therefore, according to national guidelines, the need for ethics committee approval was waived. Permission to access and analyse patient data was obtained from the Unit for the Clinical Management of Infectious Diseases and Clinical Microbiology of the University Hospital Complex of Granada, Spain.

Results

Demography and urine culture results

Out of the 1924 samples with suspicion of ITU processed during the study period, 1479 (76·9%) were from females (mean age: 44 years; range: 3 months to 98 years) and 438 (22·8%) were from males (mean age: 57 years; range: 2 months to 93 years). In seven cases (0·4%), the sex was not known, while in 11 cases (0·6%), the sample was from a pregnant woman.

One or more criteria for culture was met by 1217 samples (63·3%); among these, culture results were negative in 593 (48·7%), positive in 394 (32·4%) and mixed in 80 (6·6%), with microbiota from urogenital epithelia in 79 (6·5%), and presumptive urine pathogens in 70 samples (5·8%). Data were not available for one sample. Hence, 623 samples (51·2%) were used to evaluate the diagnostic accuracy obtained with BACT-Morph software and using fluorescence results in the bacterial channel.

Table 1 Urine culture results using the UF-1000i and BACT-Morph software at different bacteria count cut-off values

Cut-off	*N*	Missed	Urogenital microbiota	Contamination (mix of ≥3 pathogens)	Mix of two morphologies (mixed)	Single morphology
Global population						
BACT≥0	623	–	79	80	8	456
BACT≥30	609	14	76	79	8	446
BACT≥50	603	20	74	79	8	442
BACT≥100	588	35	70	76	8	434
BACT≥150	570	53	69	72	7	422
Hospital						
BACT≥0	192	–	17	19	4	152
BACT≥30	183	9	16	18	4	145
BACT≥50	180	12	15	18	4	143
BACT≥100	170	22	12	16	4	138
BACT≥150	162	30	11	15	3	133
Primary care						
BACT≥0	431	–	62	61	4	304
BACT≥30	426	5	60	61	4	301
BACT≥50	423	8	59	61	4	299
BACT≥100	418	13	58	60	4	296
BACT≥150	408	23	58	57	4	289

All data are reported as the number of cases. BACT: bacteria per μl; *N*: sample size.

Diagnostic accuracy of BACT-Morph software

Table 1 exhibits the urine culture results obtained using the BACT-Morph software of the UF-1000i analyser according to different bacterial count cut-offs.

For our classification using BACT-Morph software, 'true rods' were defined by the presence of Gram-negative rods alone or coexisting with another micro-organism with different morphology and lower count. Other results were assigned to the 'not-rod' category. Table 2 and Table S1 display the diagnostic accuracy results for the different bacterial count cut-off values studied. At the lowest cut-off (>30 bacteria per μl), BACT-Morph showed 82·4% (95% CI: 77·8–86·2) sensitivity, 62·5% (56·3–68·2) specificity, 73·5% (68·7–77·8) PPV and 73·7% (67·4–79·2) NPV for the global series (N = 609). After excluding samples with contamination and microbiota (454/609), 82·4% (77·8–86·2) sensitivity, 66·7% (57·1–75·1) specificity, 88·1% (83·9–91·3) PPV and 55·9% (47·1–64·3) NPV were obtained. Thus, the diagnostic accuracy was 72·6% for all samples and reached 78·4% when samples with contamination and microbiota were excluded. No significant differences were observed as a function of the cut-off value in the global series or in samples from the hospital or community.

Morphology analysis based on B_FSC and B_FLH values

Bacterial channel B_FSC and B_FLH values were compared between *Gram-negative rods*, i.e. *true rods* as described above, and *Gram-positive cocci*, including Gram-positive cocci alone or in the presence of other micro-organisms with lower counts. As shown in Table 3, significant differences were found between these categories in B_FSC ($P < 0{\cdot}001$) but not B_FLH values ($P = 0{\cdot}330$) with higher B_FSC values for the Gram-positive cocci.

The correct identification of Gram-positive cocci was assessed by constructing ROC curves for B_FSC fluorescence values (Fig. 1 and Table 4). In the whole series, the AUC was 0·719 for a cut-off of B_FSC ≥20·6 analytical channel (ch), providing a sensitivity of 90·0% (85·8–93·2), specificity of 38·9% (33·7–44·3), PPV of 54·8% (50·1–59·4) and NPV of 82·6% (75·7–88·0), with a diagnostic accuracy of 59·9%. In the series excluding samples with contamination or urogenital epithelial microbiota, the AUC increased to 0·755 for a cut-off of B_FSC ≥24·2 ch, with a sensitivity of 90·2% (83·1–94·6), specificity of 49·1% (43·7–54·6) and NPV of 93·3% (88·4–96·4) but reduced PPV of 38·7% (33·1–96·4), with a diagnostic accuracy of 62·0%.

Analysis of the two-dimensional scatter plot for the two fluorescence values (Fig. 2) showed a higher B_FSC/B_FLH ratio for 'Gram-positive cocci' than for 'Gram-negative rods'.

Table 2 Diagnostic accuracy for rod-shaped bacteria (*true rods*) with BACT-Morph software at cut-off of 30 bacteria per μl

	Including samples with microbiota and/or contamination					Excluding samples with microbiota and/or contamination				
	N	SE	SP	PPV	NPV	N	SE	SP	PPV	NPV
Global	690	82·4 (77·8–86·2)	62·5 (56·3–68·2)	73·5 (68·7–77·8)	73·7 (67·4–79·2)	454	82·4 (77·8–86·2)	66·7 (57·1–75·1)	88·1 (83·9–91·3)	55·9 (47·1–64·3)
Hospital	183	76·3 (67·3–83·6)	72·5 (60·2–82·2)	82·1 (73·2–88·6)	64·9 (53·1–75·2)	149	76·3 (67·3–83·6)	77·1 (59·5–89·0)	91·6 (83·6–96·0)	50·0 (36·3–63·8)
Primary care	426	85·4 (80·0–89·6)	59·0 (51·8–65·8)	70·2 (64·3–75·5)	78·2 (70·5–84·3)	305	85·4 (80·0–89·6)	62·0 (50·4–72·5)	86·6 (81·2–90·6)	59·8 (48·3–70·3)

N: sample size; SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value. All data are reported as percentages (with 95% CI).

Table 3 Relationship of bacterial channel parameters with micro-organism aetiology

	N	Mean	SD	Min	P25	Median (P50)	P75	Max
B_FSC								
Gram-negative rods	341	28·3	16·0	6·8	16·5	24·3	37·3	108·1
Gram-positive cocci	115	44·7	20·5	0·0	30·9	40·0	52·3	144·5
Two morphologies (mixed)	8	37·1	21·4	16·4	23·3	26·7	55·5	77·6
Total	464	32·5	18·7	0·0	18·3	29·1	42·0	144·5
B_FLH								
Gram-negative rods	341	106·1	28·3	70·0	83·5	100·6	119·5	207·7
Gram-positive cocci	115	104·4	20·2	0·0	92·7	101·8	112·2	176·7
Two morphologies (mixed)	8	105·0	15·0	85·5	92·0	102·9	118·1	127·8
Total	464	105·6	26·3	0·0	87·4	100·9	117·3	207·0

B_FSC: bacterial channel forward-scattered light; B_FLH: bacterial channel fluorescence-scattered light; *N*: sample size; SD: standard deviation; Min: minimum value; Max: maximum value; P25/50/75: percentiles. (excluding samples with contamination/microbiota). All data are reported as number of cases.

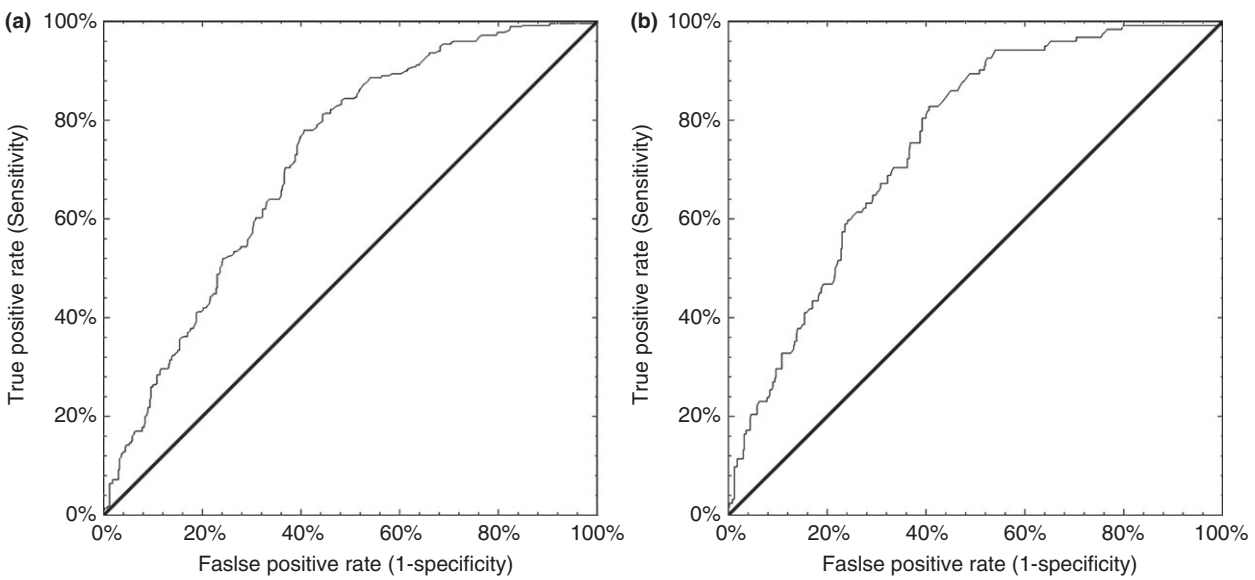

Figure 1 Receiver operating characteristics curve for prediction of cocci-shaped bacteria from B_FSC values, considering samples with counts of >30 bacteria per μl. (a) All samples. (b) Excluding samples with contamination and/or urogenital epithelia microbiota.

Table 4 Detection accuracy of 'Gram-positive cocci' urinary tract infections

Samples with microbiota and/or contamination	AUC	B_FSC cut-off	SE	SP	PPV	NPV
Included	0·719 (0·677–0·765)	20·6	90·0 (85·8–93·2)	38·9 (33·7–44·3)	54·8 (50·1–59·4)	82·6 (75·7–88·0)
Excluded	0·755 (0·705–0·798)	24·2	90·2 (83·1–94·6)	49·1 (43·7–54·6)	38·7 (33·1–44·7)	93·3 (88·4–96·4)

SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value (with 95% CI).

Discussion

The UF-1000i analyser has proven useful to screen urine samples from patients with suspected UTI (De Rosa *et al.* 2010; Wang *et al.* 2010; Broeren *et al.* 2011; Gutiérrez-Fernández *et al.* 2012; Marschal *et al.* 2012). Although the main priority in urine sample screening by cytometry is to provide a high sensitivity and NPV to rule out

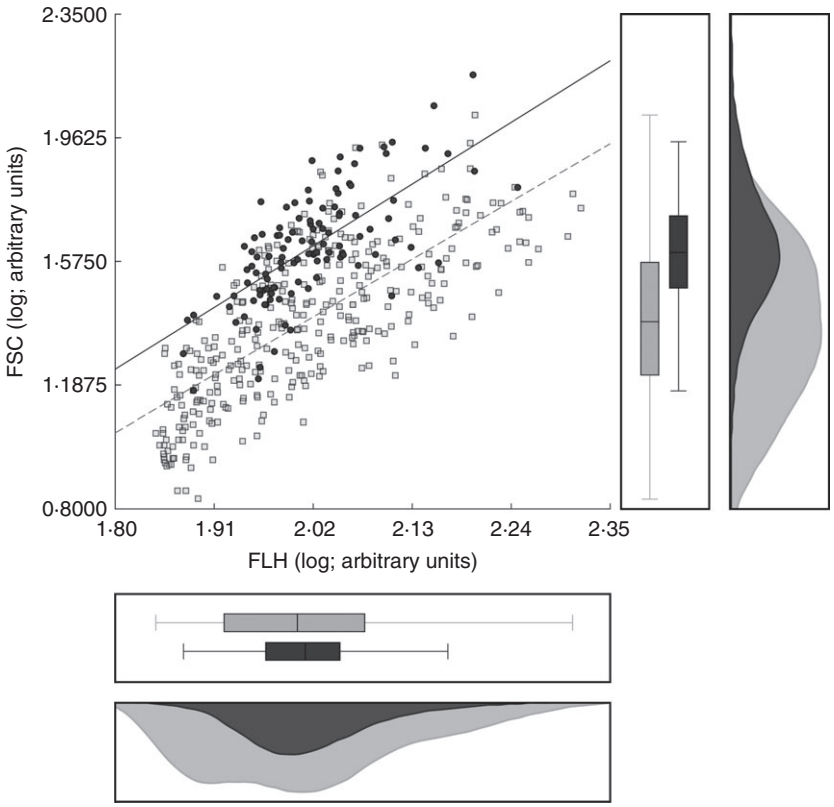

Figure 2 Scatter plot of B_FSC and B_FLH values in log scale according to pathogen morphology determined by urine culture. Black dots: B_FSC and B_FLH values of 'Gram-positive cocci' category. Black solid line: trend line of 'Gram-positive cocci' category. Grey squares: B_FSC and B_FLH values of 'Gram-negative rods' category. Grey dashed line: trend line of 'Gram-negative rods' category.

nonsignificant UTIs, bacterial channel fluorescence values yielded by UF-1000i may be useful to discriminate the morphology of the aetiological agent. The achievement of optimum predictive values could substantially reduce the time-to-diagnosis and improve the prescription of antibiotics, which is generally focused on a broad-spectrum approach against Gram-negative rods. This study examined the usefulness of the fluorescence parameters generated by Sysmex UF-1000i for the rapid diagnosis of urinary tract infection with or without BACT-Morph software.

The present results are consistent with recent reports that Gram-negative rods, mainly *E. coli*, are the most frequent pathogens in UTIs of adults from primary care (Foxman 2010; Martín-Gutiérrez *et al.* 2015). However, an increase in Gram-positive cocci or yeasts has been detected in UTIs of hospital origin (Sorlozano *et al.* 2014).

The BACT-Morph software of Sysmex UF-1000i demonstrated satisfactory diagnostic accuracy (72·6%) to predict rod-shaped bacteria but inadequate accuracy for correct cocci classification, similar to previous findings (Geerts *et al.* 2015). The two additional fluorescence parameters available, B_FSC and B_FLH, may be useful to discriminate micro-organism aetiology (De Rosa *et al.* 2010; Gessoni *et al.* 2015), but no suitable cut-off has been established for routine practice. While Gram-negative rods remain as single particles in fluids, cocci aggregation in chain structures or irregular brunches might lead to their misclassification as single particle by the analyser (De Rosa *et al.* 2010; Gessoni *et al.* 2015). The resulting increase in B_FSC values means that utilization of this fluorescence parameter for the classification of rod-shaped bacteria by flow cytometry may lead to underestimation of the count and a rise in false-negative results. In our assessment of the usefulness of bacterial channel FSC values to predict cocci morphology, we identified a cut-off value of 20·6 ch when all samples were included and one of 24·2 ch when samples with contamination of urogenital epithelial microbiota were excluded. Sensitivity and NPV values were high, but the diagnostic accuracy was relatively low (59·9% and 62·0%, respectively) for both cut-off points. In contrast, acceptable specificity and high sensitivity and NPP were obtained for both cut-offs when the B_FSC/B_FLH ratio was used, being higher in cocci than in rods, in agreement with the findings published by Geerts *et al.* (2015).

The inclusion of mixed samples or those with microbiota from urogenital epithelia modified the performance achieved using either the B_FSC parameter alone or BACT-Morph software, reducing the specificity, NPV and diagnostic accuracy. It was not possible in this study to recover data on the morphology and concentration (CFU per ml) of each species present in samples, which were classified as 'cocci/mixed' in order to obtain more

conservative results. Further prospective studies are required to assess the true impact of other variables gathered by this system, such as yeast, epithelial cells or round cells, as potential predictors of contamination.

Study limitations include the retrospective design. Urine sample culture and flow cytometer assessments were not carried out simultaneously, and the morphology and CFU per ml of mixed cultures or microbiota were not available, and these samples were subjectively assigned to the 'cocci/mixed' category. In addition, urine samples with bacteria count <30 bacteria per μl were not evaluated by cytometry, being below our threshold setting for Sysmex UF-1000i. Fluorescence values may also be biased due to the heterogeneity of patients, intrinsic risk factors and, especially, uncertainty about previous antibiotic treatment, which can affect the bacteria structure (Gessoni *et al.* 2015). Finally, it was assumed in the data analysis that the presence of two different species of Gram-negative rods or Gram-positive cocci in a single culture did not affect the fluorescence values and that the rods and cocci in a culture with nonsignificant counts did not affect rod-associated fluorescence values. Future studies should only consider samples with a single pathogen detected in culture, assigning other samples to the mixed category.

In conclusion, the Sysmex UF-1000i analyser is a useful and rapid tool to screen and diagnose UTIs. In our settings, BACT-Morph software proved suitable to identify rod-shaped bacteria in our setting, whereas the B_FSC/B_FLH ratio provided superior cocci classification. Further research is warranted to verify the utilization of this system for aetiological diagnosis.

Conflict of Interest

None of the authors have any conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Diagnostic accuracy for rod-shaped bacteria (*true rods*) with BACT-Morph software at different cut-off values