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ORIGINAL ARTICLE

Performance of the Sysmex UF1000i system in screening for significant bacteriuria before quantitative culture of aerobic/facultative fast-growth bacteria in a reference hospital

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Abstract

Objective: To evaluate the performance of the Sysmex UF1000i automatic urine screening system in the quantitative culture of fast-growth aerobic/facultative bacteria.

Methods and Results: A standard procedure was used to recover fast-growth aerobic/facultative micro-organisms in 1225 samples, applying (Sysmex®) flow cytometry for parallel bacteria and leucocyte counts. According to the area under the receiver operating characteristic curve, the optimal cut-off values to detect bacteriuria $>10^5$ colony forming units (CFU) ml^{-1} were 690/μl for bacteria and 38/μl for leucocytes (sensitivity, 92%; specificity, 65%; positive predictive value [PPV], 39%; and negative predictive value [NPV], 97%). The use of a single cut-off point of 150 bacteria μl^{-1} to detect significant bacteriuria of $>10^5$ CFU ml^{-1} or of $\geq 10^4$ CFU ml^{-1} plus leucocyturia obtained similar results (sensitivity, 89%; specificity, 54%; PPV, 31%; and NPV, 96%) and allowed 45·7% of the samples to be rapidly excluded.

Conclusions: The Sysmex UF1000i system can be adapted for bacteriuria screening by the use of an appropriate cut-off point.

Significance and Impact of the Study: This screening system significantly reduces the workload and produces very few false positives and negatives.

Introduction

Urinary tract infection (UTI) is the most frequent infectious entity in our setting and is associated with severe complications in certain population groups, often attributable to an incorrect diagnosis (Sobel and Kaye 2010). The usual aetiologies are *Enterobacteriaceae*, especially *Escherichia coli;* enterococci; *Staph. saprophyticus* (in women of child-bearing age); nonfermenting gram-negative rods; and *Corynebacterium urealyticum* (in patients with underlying urinary tract disease) (Baron and Thomson 2011).

Laboratories have a high workload for the microbiological diagnosis of UTI, which is one of the most requested studies. The reference method is to cultivate the samples in culture media to identify and quantify significant causal agents and determine their sensitivity to antibiotics. A definitive diagnosis of UTI is based on bacteriuria data along with the leucocyte count, when necessary, and the clinical characteristics of the patient. However, although leucocyturia is frequently associated with UTI, its presence may also be due to vaginal infection, inflammation without UTI or infection at a different localization (Sobel and Kaye 2010). Numerous samples are negative in culture, and an effective screening system in laboratories would avoid unnecessary cultivation of samples, thereby reducing the workload (Manoni *et al.* 2009).

Rapid information can be obtained on the presence of bacteriuria and/or pyuria by the use of reactive strips, largely for bacterial nitrate reductase and/or leucocyte esterase activity (Palacios *et al.* 2002), and by means of automated systems. Various techniques are used in these systems, including automated microscopy (IQ200), flow cytometry (Sysmex UF1000i), hybridization with fluorescent probes (Cellenium 160 US) or bacterial ATP measurement *via* enzymatic reaction (Coral UTI Screen) (Gutiérrez *et al.* 2006; Andreu *et al.* 2011). Negative findings (no significant bacteriuria) can be delivered to health system computer networks in real time by these automated screening systems. However, the criteria for positivity need to be adapted to the characteristics of a given population. Thus, distinct bacteria and/or leucocyte cut-off points have been established for screening with the Sysmex UF1000i system (TOA Medical Electronics, Kobe, Japan) in different settings (Pieretti *et al.* 2010; Rosa *et al.* 2010), and some authors even ruled out its clinical use (Broeren *et al.* 2011; Marschal *et al.* 2012). The results obtained with the same system by other authors are displayed in Table 1, evidencing the very wide variation in cut-off points applied and the sensitivity and specificity results obtained. The UF1000i system uses flow cytometry to count red blood cells, white blood cells, casts, bacteria, yeasts and epithelial cells in urine, but further studies are required to establish the value of this system and interpret the results obtained. The objective of this study was to evaluate the performance of Sysmex 1000i analyzer in the screening of urine samples for significant bacteriuria before the quantitative culture of fast-growth aerobic/facultative micro-organisms.

Materials and methods

We studied all 1225 consecutive urine samples received by our laboratory during April and May 2011 from primary and specialist care units of the Virgen de las Nieves University Hospital, a reference centre in the southern Spanish region of Andalusia. The hospital serves a population of around 440 000 individuals. In-patient samples ($n = 158$) were obtained from 27 catheterized adults (2·2% of total samples), 33 children (2·7%) and 98 immunocompromised adults (8%), and out-patient samples ($n = 1067$) were obtained from 464 adults with no clinical data of interest (37·9% of total samples) and 603 pregnant women (49·2%), as controls. All individuals were of Caucasian origin. Samples from the children were obtained by using paediatric bags. Samples from the adults were gathered by urinary catheter or by the clean-catch midstream technique, always using sterile wide-rim containers or tubes with boric acid (Vacutainer®; Becton Dickinson, Franklin Lakes, NJ, USA) (Andreu *et al.* 2011). The sterile containers/tubes were refrigerated and processed within 24 h of the sampling in accordance with a previously published procedure (Palacios *et al.* 2002). The sample was first inoculated on CHROMagar Orientation® medium (Becton Dickinson) in a quantitative manner using a 1-μl calibrated loop (COPAN, Brescia, Italy); in cases with underlying urinary tract disease, an additional 10 μl was inoculated on Columbia blood agar (Becton Dickinson). The sample was imaged by using an inverted microscope, adding 10 μl urine to a microtitre plate and recording the mean number of leucocytes per field. After 18–24 h of medium incubation in aerobic atmosphere at 37°C, fast-growth uropathogens were counted, classifying counts as follows (Pezzlo *et al.* 2010): negative (<10 000 CFU ml^{-1}); presumptive (10 000–100 000 CFU ml^{-1} of two uropathogens or one uropathogen without leucocyturia); significant (bacteriuria or candiduria with >100 000 CFU ml^{-1} of one or two uropathogens or 10 000–100 000 CFU ml^{-1} of one uropathogen with leucocyturia); or mixed (>10 000 CFU ml^{-1} of more than two uropathogens). Results for samples evidencing candiduria in culture ($n = 7$) were excluded from our analysis of the performance of the UF-1000i system.

In parallel to their cultivation on chromogenic medium, the urine samples underwent bacteriuria and leucocyturia analyses with the Sysmex UF-1000i analyzer. In

Table 1 Diagnostic yield of the cut-off points obtained in published studies

Author/year	Cut-off point	Sensitivity/Specificity	PPV/NPV
Manoni (2009)	>125 bacteria μl^{-1} and >40 leucocytes μl^{-1}	0·99/0·77	0·98/0·82
De Rosa (2010)	170 bacteria μl^{-1} and 150 leucocytes μl^{-1}	0·98/0·76	–/0·99
Jolkkonen *et al.* (2010)	Different cut-off points	0·934/0·823	0·983/–
Pieretti (2010)	65 bacteria μl^{-1} and 100 leucocytes μl^{-1}	0·98/0·62	0·98/0·53
Wang (2010)	>100 bacteria μl^{-1} or >56 leucocytes μl^{-1}	0·89/0·95	0·91/0·94
van der Zwet (2010)	50 bacteria μl^{-1} and/or 20 leucocytes μl^{-1}	0·81–1/0·54–0·80	0·92–1/0·39–0·59
Broeren (2011)	230 bacteria μl^{-1}	0·95/0·80	–/0·99
Jiang *et al.* (2011)	4000 bacteria μl^{-1}	0·54/0·96	0·56/0·96

NPV, negative predictive value; PPV, positive predictive value.

the Sysmex system, cut-off values for bacteria and leucocytes were evaluated according to the area under the receiver operating characteristic (ROC) curve, which was estimated (with standard error) by using the nonparametric method of Hanley and McNeil (1982). Two definitions of positive bacteriuria were used: CFU $ml^{-1} \geq 10^4$ and CFU $ml^{-1} > 10^5$/ml. The prevalence of positive bacteriuria in the population was calculated by using the point estimate and exact confidence interval of Clopper–Pearson. Curves were compared by the method of De Long *et al.* (1988). The cut-off point for each variable (and for each bacteriuria definition) corresponded to the value at which the sensitivity and specificity were closest (corresponding to a 45° tangent line), using the method of Farrar *et al.* (2001). Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values were then calculated for these cut-off points. We compared the diagnostic value of the cut-off points (≥ 50, 150 and 200 bacteria μl^{-1}) to detect significant plus presumptive bacteriuria or only significant bacteriuria (Manoni *et al.* 2009; Wang *et al.* 2010; van der Zwet *et al.* 2010). The Spearman test was used to compare quantitative measures (bacteria count). $P \leq 0{\cdot}05$ was considered significant. STATA Release 10.1 statistical package (StataCorp LP, Lakeway Drive, TX, USA) was used for the data analyses.

Results

Results obtained by conventional methods for the 1225 samples were (Table 2) 853 (69·6%) negative counts, 20 (1·6%) mixed counts, 117 (9·5%) presumptive bacteriuria, 228 (18·6%) significant bacteriuria and seven (0·6%) candiduria. Bacteria isolated in the 228 samples with significant bacteriuria were *E. coli* (119), *Enterococcus faecalis* (32), *Klebsiella pneumoniae* (19), *Streptococcus agalactiae* (12), *Proteus mirabilis* (9), *Pseudomonas aeruginosa* (6), *Klebsiella oxytoca* (4), *Morganella morganii* (3), *Providencia stuartii* (3), *Staph. saprophyticus* (3), *Enterococcus faecium* (2), *E. coli* and *E. faecalis* (2), *E. coli* and *K. pneumoniae* (2), and other micro-organism combinations (12).

The prevalence of bacteriuria was 29·9% (95% CI: 26·8–32) when defined as $\geq 10^4$ CFU ml and 18·9% (95% CI: 17·2–21·7) when defined as $>10^5$ CFU ml^{-1}. Table 3 lists the leucocyte and bacteria counts measured with the Sysmex system for each definition of positive bacteriuria. The mean values of both counts were higher in samples with positive bacteriuria by either definition than in negative samples ($P < 0{\cdot}001$); the differences between positive and negative bacteriuria were significantly greater in both variables (leucocyte and bacteria counts) when the stricter definition was applied ($P < 0{\cdot}01$). These two variables were significantly correlated ($P < 0{\cdot}001$, Spearman rho coefficient = 0·5632). Table 4 shows the capacity of the two variables to discriminate between positive bacteriuria and negative cultures according to the two definitions of bacteriuria; the areas under the ROC curve show that both variables discriminated well between positive bacteriuria and negative cultures ($P < 0{\cdot}001$), although the discriminating capacity of the bacteria value was higher ($P = 0{\cdot}0156$) than that of the leucocyte count when the definition of bacteriuria was less strict ($\geq 10^4$ CFU ml^{-1}), while the discriminating capacity of both variables was increased with the stricter definition ($>10^5$ CFU ml^{-1}), with no difference between them ($P = 0{\cdot}6950$). Table 5 shows the cut-off points obtained for the variables with each definition and their diagnostic yield. Table 6 exhibits the diagnostic yield obtained by combining these cut-off points.

Table 2 also shows the bacteriuria results obtained by Sysmex UF1000i at the different cut-off points (≥ 50, 150, and 200 bacteria μl^{-1}). Excluding samples with mixed counts and using a cut-off point ≥ 150 bacteria μl

Table 2 Bacteriuria detected with culture and with Sysmex UF1000i for different cut-off points

				Sysmex results					
				Bacteriuria cut-off point μl^{-1}					
Culture results	No	Bacteriuria	No	<50	≥50	<150	≥150	<200	≥200
Significant count*	232	Significant*	228	10	218	24	204	32	196
Presumptive count†	120	Presumptive†	117	37	80	54	63	57	60
Mixed count‡	20								
Negative count	853		853	334	519	469	384	506	347
Subtotal			1198						
Total	1225								

*Significant count/bacteriuria = counts >100 000 CFU ml^{-1} of 1 or 2 uropathogens; or between 10 000 and 100 000 CFU ml^{-1} of one uropathogen with leucocyturia.

†Presumptive count/bacteriuria: counts between 10 000 and 100 000 CFU ml^{-1} of two uropathogens or of one pathogen without leucocyturia.

‡Mixed count: counts >10 000 of more than two uropathogens.

Table 3 Analysis of negative and positive cultures according to the definition of bacteriuria (CFU $ml^{-1} \geq 10^4$ and $>10^5$)

Definition	Culture	Sysmex' variable	*N*	Mean	SD	1st quartile	Mean	3rd quartile
CFU $ml^{-1} \geq 10^4$	Negative	leu_uf	853	107·8	888·51	4·0	10·0	36·0
		bac_uf	853	1003·4	4223·91	23·0	104·0	574·0
	Positive	leu_uf	365	782·3	2436·81	14·0	52·5	414·0
		bac_uf	365	8535·0	14 638·78	186·0	1925·0	9846·0
CFU $ml^{-1} >10^5$	Negative	leu_uf	987	100·3	835·91	4·0	10·0	34·0
		bac_uf	987	1027·0	4101·08	25·0	111·0	633·0
	Positive	leu_uf	231	1159·0	2923·75	43·0	212·5	900·0
		bac_uf	231	12 298·0	16 667·10	780·0	4973·5	15 243·0

Table 4 Area under ROC curve for each of the two variables measured in the UF1000i system for each definition

			CI (95%) AUC-ROC		
Definition	Sysmex variables	AUC-ROC	Lim. Inf.	Lim. Sup.	Signif. *P*
CFU $ml^{-1} \geq 10^4$	leu_uf	0·7239	0·6910	0·7568	0·0156
	bac_uf	0·7619	0·7312	0·7926	
CFU $ml^{-1} >10^5$	leu_uf	0·8411	0·8109	0·8713	0·6950
	bac_uf	0·8486	0·8186	0·8785	

ROC, receiver operating characteristic.

in the analyzer, 547 samples (45·7% of 1198) would not have been inoculated, but 24 (2%) significant samples (false negatives) would not have been processed for counting, identification and antibiogram. Use of the reference method revealed that 12 of these significant samples had counts >100·000 CFU ml^{-1} (eight Gram-negative bacilli and four Gram-positive cocci), including one of the two samples of hospital origin, and 12 had counts of 10 000–50 000 CFU ml^{-1} and 5–50 leucocytes μl^{-1} (eight Gram-negative bacilli and four Gram-positive cocci); all 24 samples were obtained by the spontaneous voiding of adults. According to the leucocyte count obtained with the Sysmex system, 17 (70·8%) of the 24 samples had ≥ 40 leucocytes μl^{-1}. Among all 1198 samples (excluding candiduria and mixed samples), 205 (17·1%) had a negative bacteria count and ≥ 40leucocytes μl^{-1}.

Discussion

Urine is the most frequently received sample in the microbiology laboratory and is associated with a high percentage of nonsignificant counts (Gutiérrez *et al.* 2000), explaining the need for reliable automatic screening systems to reduce the workload and speed up the clinical response time. In this study of the Sysmex UF-1000i system, we established the optimum cut-off values of bacteria and leucocytes in our setting for the diagnosis of bacteriuria, considering two definitions (CFU $\geq 10^4$ and $>10^5$ bacteria ml^{-1}). The sensitivity was not very high for either variable (bacteria and leucocyte counts) to detect bacteriuria by either definition, although it was higher with the stricter definition, as expected (76·50% for bacteria *vs* 78·63% for leucocytes). No differences in sensitivity were found between the variables. Specificity values were slightly lower but showed a similar pattern. PPVs were not very high and were slightly lower for the stricter *vs* less strict definition, as expected; the PPV did not differ between the variables. NPVs were high because of the large percentage of negative cultures (70% $\geq 10^5$ CFU ml^{-1} and 80% with $\geq 10^4$ CFU ml^{-1}), with both variables making a major contribution to these values. When positivity was defined by a result above the cut-off point for both variables, there was an increase in specificity (to 82% for $\geq 10^4$ CFU ml^{-1} and 87% for CFU $> 10^5$) and PPV (to 57% for $\geq 10^4$ CFU ml^{-1} and 55% for CFU $> 10^5$), although this value remained low. When positivity was defined by a result above the cut-off point of at least one

Table 5 Diagnostic yield of the two variables for each definition

Definition	Sysmex' variables	Cut-off point	SE	95% CI	SP	95% CI	PPV	95% CI	NPV	95% CI
CFU $ml^{-1} \geq 10^4$	leu_uf	20	69·03	66·42–71·65	65·61	62·93–68·29	45·34	42·52–48·15	83·68	81·60–85·77
	bac_uf	380	68·36	65·74–70·99	68·11	65·48–70·74	47·08	44·27–49·90	83·84	81·76–85·92
CFU $ml^{-1} >10^5$	leu_uf	38	78·63	76·32–80·94	76·57	74·18–78·96	44·66	41·86–47·46	93·71	92·34–95·08
	bac_uf	690	76·50	74·10–78·89	75·95	73·54–78·36	43·34	40·55–46·14	93·07	91·64–94·51

Confidence interval (CI), Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV).

Table 6 Diagnostic yield of the combination of the two variables for each definition

Definition	Variables	SE	95% CI	SP	95% CI	PPV	95% CI	NPV	95% CI
CFU ml^{-1} $\geq 10^4$	leu_uf>=20 & bac_uf >=380	56·50	53·70–59·29	82·18	80·02–84·34	56·82	54·02–59·61	81·99	79·82–84·16
	leu_uf>=20 or bac_uf >=380	81·07	78·86–83·28	51·47	48·65–54·28	40·94	38·17–43·72	86·76	84·85–88·67
CFU ml^{-1} $>10^5$	leu_uf>=38 & bac_uf >=690	63·25	60·53–65·97	87·56	85·70–89·43	55·02	52·21–57·83	90·83	89·20–92·46
	leu_uf>=38 or bac_uf >=690	91·88	90·34–93·42	64·95	62·26–67·65	38·67	35·92–41·42	97·08	96·13–98·03

Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV).

variable, there was an increase in sensitivity (to 81% with $\geq 10^4$ CFU ml^{-1} and 91·9% with CFU $> 10^5$) and a major increase in the NPV, especially with the stricter definition (to 86·8% for $\geq 10^4$ CFU ml^{-1} and 97·1% for $\geq 10^5$ CFU ml^{-1}).

If it is only possible or desirable to use a single cut-off point (e.g., owing to incompatibility between computer systems and work systems), this should be sufficiently low to rule out the smallest number of significant urine samples. In our study, the use of a single cut-off point of 150 bacteria μl^{-1} meant that only 2% of the significant samples would have been missed in the screening, and none of these were from children or catheterized adults, that is, patients at risk of UTI complication. Addition of the criterion of ≥ 40 leucocytes μl^{-1} would have reduced many of the false negatives, but more samples without bacteriuria (to 17·1%) would have been unnecessarily inoculated. Around 80% of urine cultures are usually found to be negative (Falbo *et al.* 2012), compared with 71% in the present series, which may have resulted in a better predictive value for this cut-off point in our study. This underlines the need for a preliminary study of this type in every laboratory in which the system is to be implemented. Furthermore, if a single cut-off value is used, it is important for this to be noted in the laboratory report, along with an offer to cultivate the sample if requested by the clinician.

The Sysmex UF1000i urine flow cytometer permits the highly sensitive detection of small fluorescein-stained particles and provides scattergrams of major diagnostic value (van der Zwet *et al.* 2010). The system also offers the possibility to adapt the diagnostic algorithms and examine the urine screening results (e.g., NPVs) obtained when study variables are considered alone or in combination. The fact that we were able to rule out 45% of samples with a test of around 30 s each evidently represents a considerable potential workload saving and a major reduction in response time for negative results. In our laboratory, we can read manually around 30–40 plates per hour per person. The number of false negatives was very small, which is a critical issue given the possible clinical repercussions. Furthermore, in accordance with our current laboratory protocol, we would no longer perform leucocyte counts under the microscope.

The automated screening system must be compatible with hospital computer systems to ensure the real-time flow of clear error-free data to the requesting physician. The system produces a proportion of positives without clinical relevance, including contaminated samples (multiple pathogens/mixed contaminants). In addition, urine samples of ≥ 4 ml are required for automatic processing, and the system must be operated manually when smaller volumes are received. A further limitation is that the system cannot be used for haematic urine samples, which can obstruct the apparatus. Finally, it is essential to test the equipment daily, using an adequate number of control samples.

In summary, the Sysmex UF-1000i system demonstrated high diagnostic accuracy.. It therefore appears suitable for generalized urine screening in our setting and would reduce the workload and provide real-time data.

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