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Sysmex UF-1000i performance for screening yeasts in urine

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We tested the capacity of the Sysmex UF-1000i system to detect yeasts in urine by screening a total of 22 132 urine samples received for culture in our microbiology laboratory during 1 year. We also analyzed different dilutions of previously filtered urine inoculated with a strain of *Candida albicans*. With clinical samples, a single cut-off point of 50 yeast-like cells (YLCs)/μL detected candiduria ≥10 000 colony forming units (CFU)/mL and >100 000 CFU/mL with a sensitivity of 87.3%/95.4%, a specificity of 97%, a negative predictive value of 95.9%, and a positive predictive value of 9.3%/5.7%. With the simulated samples, a linear relationship was observed between the dilution factor and the number of cells detected by UF-1000i. This instrument appears to be able to reliably rule out candiduria of a magnitude of at least 10 000 CFU/mL and facilitate urine sample screening, thereby providing fast results. The Sysmex UF1000i system can be adapted for candiduria screening by the use of an appropriate YLCs/μL cut-off point that takes account of the prevalence of candiduria in the population.

Key words: Urine; automated system; screening; yeast; *Candida albicans*.

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The presence of yeasts in urine can be clinically relevant in certain patient groups. Hospitalized patients, elderly, and various other patient groups have shown a decreasing percentage of urinary tract infections (UTIs) produced by *Escherichia coli* and an increasing percentage produced by various agents, including *Candida albicans* and other yeasts. The underlying conditions include catheterization or instrumental manipulation of the urinary tract, cancer, and long-term antibiotic treatment (1).

Yeasts are reported to be responsible for UTIs in 18% of catheterized patients and in 1% of severely ill patients (1). Therefore, the diagnostic laboratory should be able to detect their presence in a simple manner, including effective quantification.

Our laboratory recently incorporated the Sysmex UF-1000i system (TOA Medical Electronics, Kobe, Japan) (2) to screen urine samples quantitatively before plating on a solid medium. The system uses fluorescence flow cytometry, based on diode laser technology together with hydrodynamic focusing conductometry, to count cell elements per μL of urine, including red blood cells (RBCs), leukocytes, epithelial cells, granulose cylinders, crystals, bacteria, and yeast-like cells (YLCs). For each type of particles, the scattered light is detected by a photo diode at two different positions and converted into electric signals,

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and the fluorescence intensity is measured (3). In our setting, values ≥150 bacteria/μL have proven appropriate for the detection of bacteruria in the screening of urine samples (4).

Flow cytometry has for a long time been considered useful for yeast quantification, (5) however, there is no recommended cutoff for YLCs/μL that pertains to the screening of urine samples with the Sysmex UF-1000i systems, mainly because of the capacity of YLCs to colonize the urinary tract. Marschal et al. (6) recently compared the capacity of culture vs automated methods (including Sysmex) to detect yeasts in clinical samples, but they did not recommend specific cut-off points for candiduria screening. Hence, further data are required regarding the utility of automated systems in screening for candiduria. The objective of this study was to analyze the performance of Sysmex UF-1000i in the detection of YLCs in urine and assess the reliability of a pre-selected cut-off point for the screening of urine samples prior to culture.

MATERIALS AND METHODS

The capacity of the system to detect YLCs (as candiduria marker) in urine was tested with all clinical samples received by our laboratory between September 2011 and August 2012 from primary and specialist care units of the Virgen de las Nieves University Hospital as well as with artificial samples in which yeasts were the only particles present. The Virgen de las Nieves University Hospital is a reference center in Andalusia (southern Spain), serving a population of around 440 000 individuals.

Study of YCL detection capacity in clinical samples

During the 12-month study period, 22 132 urine samples were processed following the established laboratory protocol, including screening with the Sysmex UF-1000i system. Out of these samples (4917 from males, 17 215 from females), 3886 were of hospital origin, 18 123 of community origin, and 123 of unknown origin; 1319 were from immunosuppressed patients, 6891 from pregnant women, 1288 from catheterized patients, and 344 from infants. Samples from the infants were obtained by using pediatric bags, and samples from the remainder 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). These were refrigerated and processed within 24 h of sampling following a previously reported procedure (1). Samples detected as positive by Sysmex [cutoff of ≥150 bacterial particles/μL (4) or ≥50 YLCs/μL] were plated on CHROMagar Orientation® medium (Becton Dickinson) using a 1-μL calibrated loop (COPAN, Brescia, Italy) and, in cases of clinical nephropathy, also on Columbia blood agar (Becton Dickinson). As very few data are available on YCL cut-off points, we selected half the value proposed by Wang et al. (7) to minimize the number of false negatives. Counts of uropathogenic microorganisms were classified following Pezzlo et al. (8) as negative [<10 000 colony forming units (CFU)/mL], presumptive (10 000–100 000 CFU/mL of two uropathogens or of one without leukocyturia), significant (bacteriuria or candiduria with count >100 000 CFU/mL of 1 or 2 uropathogens or 10 000–100 000 CFU/mL of 1 uropathogen with leukocyturia), or mixed flora (≥10 000 CFU/mL of >2 uropathogens).

We determined the diagnostic value of a cutoff of ≥50 YLCs/μL to detect significant candiduria, calculating the sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values.

Study of YLC detection capacity in artificial urine samples

Artificial urine samples were prepared as follows: urine from a healthy individual (JGF) was filtered through a 0.22-μm pore membrane and *C. albicans* strain ATCC10231 was then added (≈10^8 CFU/mL), followed by successive dilutions of 1/10, 1/30, 1/60, 1/180, and 1/640. Samples were then processed in duplicate according to our routine laboratory workup using Sysmex UF-1000i. The mean cell value was used in the evaluation. Subsequently, the samples were also plated on Columbia blood agar medium (Becton Dickinson), using a 10-μL calibrated loop (COPAN), followed by incubation for 48 h at 37 °C for colony enumeration.

RESULTS

Study of YLC detection capacity with clinical samples

Table 1 exhibits the YLC results obtained in clinical urine samples using the Sysmex system with a cut-off value of ≥50/μL. The system showed a reasonable diagnostic performance with the exception of a low PPV to detect counts ≥10 000 CFU/mL. The Sysmex system reported counts ≥50 YLCs/μL for 669 samples with a yeast count of <10 000 CFU/mL on

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Table 1. Detection of yeast-like cells (YLCs) in urine with the Sysmex UF1000i using a cut-off value of 50 YLCs/μL

Candiduria results CFU/mL	YLC counts		Total	Diagnostic yield			
	≥50/μL	<50/μL		Se	Sp	PPV	NPV
<10 000	669	21 384	22 053				
≥10 000	69	10	79	87.3	97	9.3	99.9
>100 000	42	4	46	95.4	97	5.7	99.9

Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; CFU, colony forming units.

CHROMagar Orientation® medium at 24 h. It recorded counts <50 YLCs/μL for 10 samples (0.047%) with yeast counts ≥10 000 CFU/mL (4 pregnant women, 5 adults with urinary bladder catheters, and one 5-month old child); YLCs/μL values ranged between 0 and 49 (mean of 11 ± 19.5). In these 10 samples, the Sysmex bacterial count was ≥150/μL, leukocyturia (≥40/μL) was detected in all cases, except for one of the pregnant women, and the bacterial count was only significant for the infant (candiduria of 10 000–100 000 CFU/mL). Finally, the four cases with a count >100 000 CFU/mL all corresponded to non-*C. albicans* yeasts.

Study of YLC detection capacity in artificial urine samples

Table 2 shows the values obtained with the Sysmex system (YLCs/μL, RBCs/μL, and total cells) and by culture (CFU/mL) of the different urine sample dilutions. The system was able to detect low yeast counts, although around 40% of yeast cells were identified as RBCs, which was reported as an anomalous result. As depicted in Fig. 1, a linear relationship was observed between urine sample dilution and YLCs values (YLCs = 12.49 + 9436 × dilution), with a slope that significantly differed from zero ($p < 0.00001$) and a correlation coefficient (r) of 0.9995. Similar results were obtained for total count values ($r = 0.9997$).

Table 2. Values obtained with the Sysmex UF-1000i system and by culture (CFU/mL) with dilutions of artificial urine samples

Dilution of urine sample	Value obtained in culture	Mean value measured by the Sysmex UF-1000i system/μL		
	CFU/mL	YLCs	RBCs	Total cells
1/10	$>10^5$	954	560	1514
1/30	$>10^5$	342	188	530
1/60	4×10^4	151	98	249
1/180	16×10^3	68	47	115
1/640	4×10^3	30	20	50

CFU, colony forming units; YLCs, yeast-like cells; RBCs, red blood cells.

DISCUSSION

The presence of yeasts in urine can only be definitively ruled out by culture (9), but automated urine screening systems may be useful in certain population groups. These systems must demonstrate high sensitivity and NPVs before they can be applied in routine laboratory work. Among the large number of clinical samples analyzed in the present study, 79 had a yeast content ≥10 000 CFU/mL. The prevalence of yeasts in the clinical samples tested was low (0.4%; 79/22132), as expected, because >80% of samples were of community origin, and the frequency of candiduria in community samples is widely considered to be <1% (10). A large majority (87.3%) of these were detected by Sysmex, but the PPV was low, which may be due either to the very low prevalence of significant candiduria in the study population or to the confusion of RBCs and leukocytes with YLCs in scattergrams (see below), or it may be that these were truly samples with candiduria <10 000 CFU/mL and therefore of doubtful clinical significance. In our work, this low PPV may not pose a problem, because the presence of candiduria frequently coincides with a bacterial count ≥150/μL, meaning that the sample would be plated on a culture medium irrespective of the detection of YLCs. Furthermore, false-nega-

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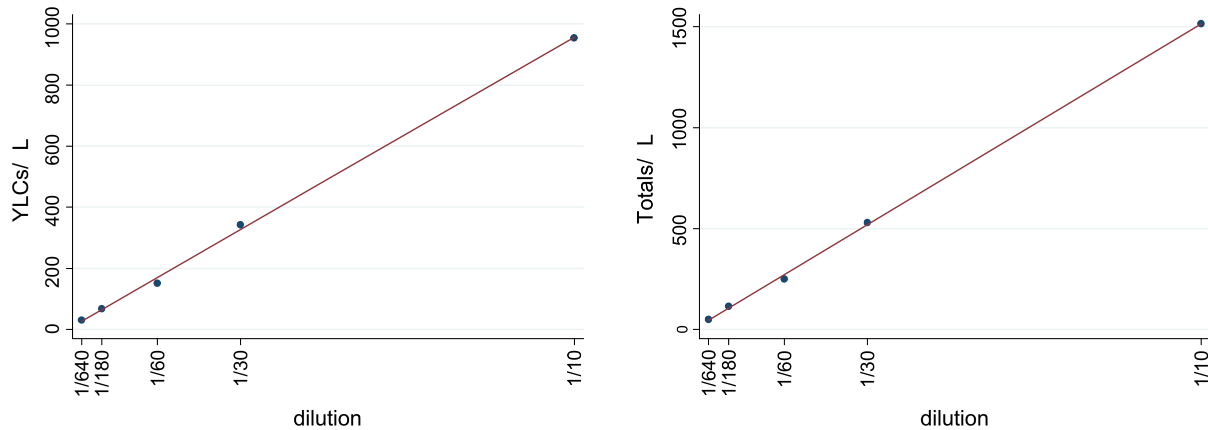

Fig. 1. Relationship of sample dilution with yeast-like cells (YLCs) and total cell values.

tive culture results cannot be ruled out, because non-*C. albicans* yeasts can grow more slowly than *C. albicans* on CHROMagar Orientation® and require more than 24 h (11), which is the standard time period established in our laboratory protocol.

A further possible limitation is that we do not know whether any of the patients were receiving antifungal drug therapy, which would result in very low yeast loads. Nevertheless, the present results for this Sysmex system are superior to those reported by Marschal et al. (6), who provided little information on their candiduria screening methodology. We have found no report offering a more exhaustive evaluation of the detection of yeast cells with this system than is provided in the present investigation. However, these findings need to be confirmed by further studies of larger numbers of samples.

In our study of artificial samples, Sysmex UF-1000i was able to detect yeasts in urine at concentrations of $\geq 4 \times 10^3$ CFU/mL. The con fusion between yeast cells and RBCs was not only reported automatically by the system but can also be considered a minor error, given that the RBC content is widely considered to be less important in microbiological urine screening (12). Van der Zwet et al. (13) used Sysmex UF1000i and obtained a good linearity of detection in samples with between 10^4 and 10^6 CFU/mL of *C. albicans*, although the presence of other particles was not reported. However, they failed to detect lower concentrations and stated that the higher concentrations were, in part, incorrectly identified as RBCs in the scattergrams. Conversely, Manoni et al. (14) studied the influence of yeasts on RBC counts and concluded that high RBC counts in urine may, in part, be attributable to the presence of yeasts. According to the above evidence, the erroneous interpretation of yeasts and RBCs by Sysmex cannot be ruled out. In contrast, Wang et al. (7) found that YLCs/μL counts between 100 and 400 did not interfere with the detection of RBCs in urine. We highlight the elevated number of total cells detected with respect to the initial inocula, reflecting the inclusion of both viable and non-viable cells. No studies have reported any interference or confusion between leukocytes and yeasts in urine samples. Finally, according to our results, a cut-off point somewhat higher than 50 YLCs/μL may be more useful for candiduria screening with the UF-1000i system in our setting, because it would increase the PPV in populations with a low prevalence.

The precision of the linear relationship between the Sysmex read-out and dilutions of artificial urine samples was very high in this study, which can be attributed to the intrinsic quality of the measurement technique in this dilution range. In fact, similar results have been obtained by other authors in comparable situations (7, 14).

In conclusion, application of the Sysmex UF-1000i system may be useful for the yeast screening of urine samples when the cut-off point is appropriate to the prevalence of candiduria in the population.

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