

SHORT REPORT

Testing by Sysmex UF-100 flow cytometer and with bacterial culture in a diagnostic laboratory: a comparison

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A large proportion of the samples tested in routine diagnostic microbiology laboratory are urine samples. The gold standard is bacterial culture, but a high proportion of samples cultured are negative. Unnecessary testing can be reduced and an improved service provided by an effective screening test. The Sysmex UF-100 flow cytometer has been developed to count cells and casts accurately in urine samples. Its performance in a screening test was compared with bacterial culture by using 1005 consecutive urine samples, and cut-off criteria were established. Cut-off values of 3000 bacteria/ μl and 111 WBC/ μl provided the best discrimination. Of 1005 samples, 606 (60%) would be cultured. Sixteen samples that were not selected according to these criteria were culture positive. This was considered acceptable for our routine use. The use of a testing algorithm incorporating the Sysmex UF-100 flow cytometer has improved the quality and efficiency of urine testing within the routine microbiology laboratory.

Urinary tract infection is common within community and hospital populations. About 50% of women state that they have experienced one infection in their lifetime and 27–48% have had recurrent infections.¹ Catheter-associated infections account for 40% of all infections acquired from hospitals.² Therefore, urine samples constitute a large proportion of the samples tested by a routine microbiology laboratory. The gold standard is bacterial culture, which is based on bacterial counts.³ Although much time and effort are spent on the processing of urine samples by microscopy and culture most samples will yield no growth.³ Methods for selecting urine samples for culture to improve the efficiency of handling these samples in the laboratory were therefore developed. Examples of selective methods include the use of reagent strips and, more recently, the development of automated methods for detection and enumeration of white blood cells (WBC), red blood cells, epithelial cells, casts and micro-organisms in urine.³ The aim of this study was to evaluate the Sysmex UF-100 flow cytometer (Sysmex Corporation, Kobe, Japan)⁴ as a means of replacing routine microscopy and reducing the number of urine samples requiring culture.

MATERIALS AND METHODS

A total of 1005 consecutive urine samples in boric acid were tested in parallel by manual microscopy and routine culture and by the Sysmex UF-100 flow cytometer from March to April 2004. The urine samples were from hospital and general practice patients and represented all age groups. Manual microscopy for WBC and red blood cells was carried out on a 2- μl urine sample by using an in-house calibrated hanging drop method based on the Neubauer chamber counting method.³ Cells were counted within one complete grid

reference square of known area and depth and multiplied by a factor of 10 to equate to the number of cells/ μl . Results were reported as <30 cells/ μl , the absolute number or >1000 cells/ μl . Semiquantitative culture was performed on one half of a culture plate of Chromogenic UTI clear agar (Oxoid, Basingstoke, UK) using a standard 2- μl loop and incubated overnight at 37°C in air. Culture results were reported as no growth, no significant growth ($<10^4$ colony-forming units (cfu)/ml), growth at 10^4 – 10^5 cfu/ml, or $>10^5$ cfu/ml. Single organism growth of $\geq 10^4$ cfu/ml was identified (Vitek 1, Biomérieux, UK) and antibiotic sensitivities were determined (Vitek 1, or by the Clinical Laboratory Standards Institute method).

The Sysmex UF-100 flow cytometer is a second-generation automated urine analyser. Details of the principles of the method have been published previously.⁵ The analyser can process 100 samples/h and is operated by trained medical laboratory assistants. The manufacturer's cut-off values for bacteria and WBC were 8000 and 111/ μl , respectively, below which urinary tract infection is deemed unlikely and culture not required. Sensitivity, specificity, positive predictive value and negative predictive value for a range of cut-off values for bacteria and WBC were determined. The number of urine samples that would be recommended for culture at these cut-off values was also determined. Ethics approval was not required for this study.

RESULTS

Table 1 shows the results of the Sysmex UF-100 flow cytometer test using a constant WBC count (111/ μl) and a variable bacteria count as the cut-off compared with bacterial culture. Forty-one (96%) of 427 samples with positive cultures were not flagged for culture by the Sysmex UF-100 flow cytometer when the manufacturer's cut-off values were used. At a cut-off value of 3000/ μl for bacteria, the number of false-negative Sysmex UF-100 flow cytometer results was 16. The culture results for these false-negative samples were *Escherichia coli* (one), coliform (four), *Streptococcus* spp/*Enterococcus* spp (four), *Staphylococcus* spp (one), yeasts (one) and mixed growth (five). The Sysmex UF-100 flow cytometer flagged the sample containing yeasts and so it would have been cultured routinely. A negative predictive value of 96% allowed for the reporting of negative results without culture being performed. This reduced the turnaround times for these samples from 24 h to same-day reporting.

Reducing the WBC cut-off to 50 or 20 WBC/ μl at 3000 bacteria/ μl lowered the number of false-negative Sysmex UF-100 flow cytometer results by two or four, but increased the number of culture plates to be set up by 21 or 72, respectively. Five (0.5%) samples were not read by the Sysmex UF-100 flow cytometer and required the use of the manual microscopy method to produce a WBC or red blood cell count. These samples were either very small or turbid.

Abbreviation: WBC, white blood cells

Table 1 Comparison of Sysmex UF-100 flow cytometer and bacterial culture results using variable bacterial counts as the cut-off values

	Cut-off value (number of bacteria/ μ l)							
	8000	7000	6000	5000	4 000	3 000	2 000	1 000
Number of culture positive/Sysmex positive	265	268	269	275	281	290	297	304
Number of culture negative/Sysmex positive	162	176	198	223	263	316	420	591
Number of culture negative/Sysmex negative	537	523	501	476	436	383	279	108
Number of culture positive/Sysmex negative	41	38	37	31	25	16	9	2
Sensitivity	86	87	88	90	92	92	97	99
Specificity	77	75	72	68	62	55	40	15
Positive predictive value	62	60	56	55	52	47	41	34
Negative Predictive value	93	93	93	94	94	96	97	98
Number of urine samples flagged for culture, total (%)	427 (42)	444 (44)	467 (46)	498 (49)	544 (54)	606 (60)	717 (71)	895 (89)

Nearly one third (31.4%) of the samples were culture negative/Sysmex positive. Some reasons for this discrepancy may be antibiotic treatment before sampling, inhibitory substances in the urine and fastidious organisms. Also, samples with pyuria will be Sysmex positive but the pyuria may not necessarily be caused by an infection.

DISCUSSION

Variable cut-off values for bacteria have been reported for use with the UF-100 flow cytometer compared with the manufacturer’s recommendations (173–8000 bacteria/ μ l).⁵⁻⁷ This study identified cut-off values for bacteria (3000/ μ l) and WBC (111/ μ l) as giving a reasonable balance between an acceptable negative predictive value (96%) and reducing the number of urine samples requiring culture by 40%. This cut-off is similar to a smaller study by Koken *et al*⁶ of urine samples from 260 patients, with a bacteria cut-off of 3800/ μ l. Of the 16 false-negative results in this study, yeast was isolated from one sample that was flagged by the flow cytometer and the remaining 15 had low WBC counts (<40/ μ l) by microscopy. Of these, five were mixed growths.

A testing algorithm was established once the appropriate cut-off values for bacteria and WBC were determined (fig 1). All suprapubic aspirates and ureteric samples are cultured regardless of the bacteria and WBC counts because the presence of any isolate may be considered as clinically relevant.⁸ Similarly, samples from patients who are potentially neutropenic (haematology/oncology wards) are

routinely cultured. Samples from children aged 5 years or under are routinely cultured because of the potential for lasting renal damage associated with urinary tract infection in this age group.⁹

Culture remains the gold standard for the detection of urinary tract infection in urine samples. The demands of human and financial resources within the microbiology laboratory, however, have meant that alternative methods have had to be introduced. The adoption of this method has improved the efficiency and quality of the service, improved turnaround times in that 40% of the urine reports are issued rapidly and released staff for other tasks.

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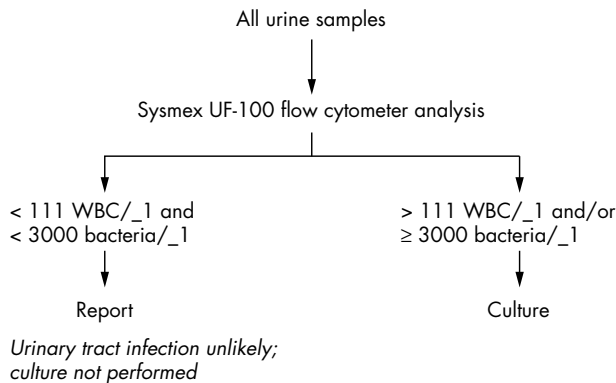


Figure 1