Use of flow cytometry (Sysmex® UF-100) to screen for positive urine cultures: in search for the ideal cut-off

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Abstract

Background: Cultures for urinary tract infections (UTI) constitute a large workload in the clinical microbiology laboratory, although up to 80% are usually negative. Several automated methods are available to screen urines for UTI, one being the flow cytometry-based Sysmex® UF-100.

Methods: The performance of the UF-100 was evaluated over a 16-month period using urine culture as the reference method.

Results: During this period, a total of 5356 urine samples were studied (469 children; 3229 women and 1658 men), of which 706 were culture positive (593 grew Gram negative bacilli). Receiver operating characteristics (ROC) curve analysis showed an area under the curve (AUC) of 0.83 for leukocytes and 0.85 for bacterial count. Applying cut-off values reported in the literature gave sensitivities ranging from 75% to 90%, resulting in 73–174 false negatives (FN). Using a logical combination (leukocytes $\geq 15 \times 10^6$/L OR bacteria $\geq 500 \times 10^6$/L) gave a sensitivity of 98%. However, the specificity dropped to 25%, resulting in 15 FN.

Conclusions: Screening urine samples for UTI detects a large number of culture positive samples. However, the rather large number of FN observed precludes the use of the UF-100 as a routine screening method to exclude urine samples from culture.


Keywords: flow cytometric screening; Sysmex® UF-100; urine culture.

Introduction

Urine culture is of the most frequent culture analysis performed in the clinical microbiology laboratory. However, 70%–80% of cultures are negative (1, 2). In the author’s institution, ~26,000 urine cultures were performed during 2008, 19% of which were culture positive. Negative urine cultures lead to considerable workload and consumption of resources, an important aspect in times of ever increasing cost restraints. This has led to the development and implementation of screening methods to identify urinary tract infections (UTI). One of the first automated methods for urinary sediment testing was based on image analysis (3, 4). However, these systems still require an operator to visually inspect images of cells and casts (5). Around the same time, improved urinary test strips and instruments for reading them, were being developed (6). Screening for UTI was based on the detection of nitrite and leukocyte esterase (7, 8). However, false negative (FN) and/or false positive results were too frequent, precluding use of this approach as a screening method for UTI (9, 10).

Recent approaches based on flow cytometry allow detection and counting of large numbers of particles in a short period of time, including bacteria and yeast (6). One such instrument for this purpose is the Sysmex® UF-100. Several studies reported acceptable performance for this instrument (6, 11–13). However, reported results for sensitivity and specificity varied significantly, with some showing a rather high number of FN results (1, 2). For example, some studies used higher cut offs, e.g., $300 \times 10^7$ bacteria/L (11, 12), in an attempt to increase the specificity to $>60\%$, while maintaining sensitivity above 90%. However, it appears that most or all of the samples were not from hospital acquired UTIs (11, 12).

The goal of this study was to evaluate the Sysmex® UF-100 system to screen urine for UTI under routine conditions in an unselected population at a major tertiary referral centre.

Materials and methods

The study was performed and approved within the ethical framework of the hospital. The study includes data from inpatients and outpatients that had a urine culture and a flow cytometric urine screen (Sysmex® UF-100, TOA Medical Electronics/Europe GmbH, Hamburg, Germany) performed between January 2008 and June 2009. Urine was collected into a Urine-Monovette® (Sarstedt, Nürmbrecht, Germany) containing boric acid for urine culture, while Urine-Monovette® tubes without boric acid were used for the flow cytometric and dipstick analysis. Only those samples were considered where both Monovette® tubes were logged into the Laboratory Information System of the Clinical Chemistry and Microbiology Department $<1$ h apart.

Dipstick urinalysis was performed using the automated Urisys® 2400 (Roche Diagnostic Systems, Lisbon, Portugal).

Manual microscopy sediment analysis was performed according to CLSI GP 16-A guideline (14). Each sample was centrifuged at 400 g for 10 min, the supernatant was decanted and the sediment mixed in the residual volume. At a $400 \times$ magnification, 20 random...
microscopic fields were examined and the mean number of cells per field were calculated.

Urine cultures were performed by inoculating well mixed, uncentrifuged urine on horse blood agar and MacConkey agar plates (bioMérieux, Lisbon, Portugal). Growth was assessed after overnight incubation at 36°C. Urine cultures were considered negative if no growth occurred or with growth <10^5 CFU/L. If more than two types of bacteria grew, samples were considered contaminated. If growth of a pure culture occurred, samples were considered positive in the presence of leukocyturia (at least 5 leukocytes/microscopic field). However, in certain patient groups, such as children, immunocompromised, transplant recipients or pregnant women, growth of a pure culture was considered positive, even in the case of <5 leukocytes/microscopic field. Microorganisms were identified by conventional biochemical tests using the WalkAway®96 system (Dade Behring, Marburg GmbH, Germany) and/or the VITEK system (bioMérieux, Lisbon, Portugal).

For flow cytometric urine screening, the Sysmex® UF-100 was used. This system analyzes formed elements in urine, including cells, based on flow cytometry and impedance detection as described previously (15, 16). In cases where patients had multiple samples processed, only the first sample was considered. Samples that were considered to represent bacterial contamination were excluded. The culture results were used as the reference method to evaluate the performance of the Sysmex® UF-100 as a screening method for UTI.

Data were analyzed using SPSS Statistics V17.0 (SPSS Inc, Chicago, IL, USA).

Results

A total of 5356 (4650 culture negative and 706 culture positive) urine samples fullfilled the inclusion criteria. Of these, 469 were from children <16 years (mean: 6 years, range: 2 days–15 years), 3229 from female patients (mean: 50 years, range: 16–100 years) and 1658 from male patients (mean: 62 years, range: 16–97 years). Bacteria that were isolated are shown in Table 1.

Table 1 Bacterial isolates from positive urine cultures (n=706).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Children (≤16 years)</th>
<th>Adult, male</th>
<th>Adult, female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative bacilli</td>
<td>49</td>
<td>140</td>
<td>404</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>26</td>
<td>78</td>
<td>287</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>2</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>8</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>10</td>
<td>47</td>
<td>56</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>0</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>7</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>3</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>187</td>
<td>460</td>
</tr>
</tbody>
</table>

Other = Morganella morganii; Serratia spp.; Citrobacter spp.; Enterobacter spp.; Providencia spp.; Stenotrophomonas maltophilia.

If no growth occurred or with growth <10^5 CFU/L OR Bacteria ≥500×10^5/L, which produced a sensitivity of 98%, but a specificity of only 25%, resulting in 15 FN. Of these FN, two were from Obstetrics and three from the Nephrology Department. Underlying conditions most frequently associated with FN were renal transplanta-

Discussion

Urine cultures constitute a major part of the clinical microbiology workload. Considering that 70%–80% of urine cultures are usually negative (1, 2), a screening test that reliably identifies samples from patients with a UTI would have a major impact, and large cost savings could be achieved. The appearance of flow cytometric urine analysis seemed to provide such a method. However, such a screening method would need to perform well in uncomplicated UTIs. Many cases of uncomplicated UTIs, for example in young, sexually active women, are likely treated empirically, without resorting to urine culture (17). However, complicated UTIs often associated with complex underlying pathologies, such as immunosuppression, are often caused by hospital-related, antibiotic resistant strains (17). For these infections, a screening tool for UTI would have to offer reliable detection when used in a large hospital setting. In this context, it is notable that some of the studies that evaluated the Sysmex® UF-100 excluded hospital acquired infections (12).

Figure 1 ROC curve for leukocyte and bacterial counts (n=5356).

Receiver operating characteristics (ROC) curve for bacteria and leukocyte counts on the Sysmex® UF-100 flow cytometer, using urine culture as the reference method. The area under the curve (AUC) is 0.83 for leukocytes and 0.85 for bacterial counts (0.5–0.7: low accuracy, 0.7–0.9: moderate accuracy and >0.9 high accuracy). ROC curve generated with SPSS Statistics V17.0.
Although the Sysmex® UF-100 detects a large percentage of UTIs, reported sensitivities are only in the range of 70%–90% (1, 2, 15, 18). However, some investigators reported higher sensitivities (>95%), but this caused the specificity to drop to values as low as 15%–49% (11, 12). Interestingly, the cut-offs that were used in these studies varied widely (Table 2) (11, 12, 15, 18). When these cut-offs were applied in the current study, the best sensitivity did not exceed 90%, with a rather low specificity of 57%. To achieve the best cut-off, a logical combination of a low leukocyte count (≥15 × 10^6/L) and bacterial count (500 × 10^6/L) was used (Table 2). However, this resulted in a large number of false positives as the specificity decreased to 25%. Extrapolating these results to 26,000 urine cultures/year that our laboratory processes, with a 19% positive rate, translates into 99 FN (2%) and 5265 negative urine cultures that would be avoided. Interestingly, some studies used 300 × 10^6 bacteria/L as the cut-off, with good results for sensitivity (~95%) and specificity (>60%) (11, 12). However, these studies included a much smaller number of cultures. For example, Kim et al. included 330 urine cultures and excluded hospital-acquired infections (12). In addition, one study reported 16 FN out of a total of 427 positive cultures (11). This illustrates the importance of investigating the usefulness of this method for screening for all types of UTIs.

It has already been reported that most FN results are observed primarily in certain patient groups, including those that are immunosuppressed, renal transplant recipients or pregnant woman (2). Of course, it is possible that in some of these cases, microbiologists “over” interpret the urine culture and consider them significant. However, rigorous application of criteria, such as the leukocyte count or bacterial growth would not support this interpretation. Such urine specimens most likely would show a negative screening result using the Sysmex® UF-100. Still, one could use the screening method to exclude unnecessary urine culture specimens, applying a high cut-off sensitivity of 98% or 99%, and accepting low specificity. For example, applying a sensitivity of 98% in the authors’ hospital, 5364 cultures could have been avoided in 2008. Even so, this approach would have missed 99 positive urine cultures (FN), an acceptably high number.

In conclusion, the Sysmex® UF-100 is a useful method for screening urine samples for UTI. However, clear cut-off values are not readily available and are difficult to establish. For this method to be a useful screening tool for UTI in the hospital setting, the FN rate would have to be acceptably low. This, possibly, would require instrument performance with sensitivity >99% to ensure that clinically important cases of UTI would not be missed.

### Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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References


