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# Relationship between conventional culture and flow cytometry for the diagnosis of urinary tract infection

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ABSTRACT

*Background:* Urine culture is the gold standard for the diagnosis of urinary tract infections (UTI). The use of flow cytometry analyzers (FCA) prior to culture allows for the quantification and recognition of cell components in urine to be automated and makes it possible to relate these data to the urine pathogens subsequently identified in cultures.

*Methods:* Urine samples were assessed with the Sysmex UF-1000i analyzer. Those that met the criteria for culture (> 25 leukocytes/μL or > 385 bacteria/μL) were subjected to quantitative urine culture on chromogenic agar. Counts of red blood cells (RBC), white blood cells (WBC), epithelial cells (EC), and the kind of microorganisms identified in cultures were evaluated.

*Results:* A total of 17,483 samples were processed by FCA. Of these, 9057 met the criteria for culture. Urine cultures were reduced by 48.2%. The most common urine pathogen was *Escherichia coli* (60.3%). Negative urine cultures were significantly ($p < 0.001$) associated with a lower WBC count than urine with *E. coli*, *Klebsiella* spp. and *Proteus spp.*, but urine with *Enterococcus* spp. had a lower WBC than negative urine. Contaminated urine had a significantly ($p < 0.001$) lower WBC than urine with *E. coli*, *Klebsiella* spp. and *Proteus spp.*, but no differences were found for *Enterococcus spp.* ($p = 0.729$). Negative urine cultures had significantly ($p < 0.05$) higher EC than all positive urine samples. Contaminated urine was associated ($p < 0.001$) with higher EC than cultures with *E. coli* and *Klebsiella spp.*, in comparison with cultures with *Enterococcus spp.* ($p = 0.091$) and *Proteus spp.* ($p = 0.251$).

*Conclusion:* The use of the Sysmex UF-1000i flow cytometer for screening urine samples allows for a reduction in the number of urine cultures. WBC values correlate well with the main urine pathogens related to UTI. The results observed for *Enterococcus* spp. suggest a low impact of these pathogens as a cause of UTI.

## 1. Introduction

Urinary tract infection (UTI) is one of the most common infections in humans (Foxman, 2010), with urine being the most frequent sample that is submitted for culture examination in clinical microbiology laboratories (Muñoz-Algarra et al., 2013). UTI typically exhibits bacteriuria and pyuria, and urine culture is the gold standard for etiological diagnosis (Giesen et al., 2013; Medina-Bombardó and Jover-Palmer, 2011; Schmiemann et al., 2010), despite its long turnaround time (24 to 48 h). The screening of urine by flow cytometer analyzers (FCA) can lead to a reduction in urine cultures and faster reporting of negative results, avoiding unnecessary antibiotic treatment (de Frutos-Serna et al., 2014; Gómez-camarasa et al., 2015). Furthermore, the examination of urine with FCA prior to culture can improve the diagnosis by correlating FCA parameters, such as the presence of white blood cells (WBC), red blood cells (RBC) and epithelial cells (EC), with the kind of microorganisms isolated in urine samples from patients with a suspected UTI (Monsen and Rydén, 2015). The Sysmex UF-1000i analyzer is a flow cytometer analyzer equipped with a laser and two analysis channels, one for bacteria and another one for other urine forming elements. Using a fluorescent dye that stains DNA, the analyzer counts particles based on internal and external structures, size and fluorescence (Giesen et al., 2013; Moshaver et al., 2016). Use of the Sysmex UF-1000i analyzer for screening urine to rule out suspected UTI is feasible according to several previous studies (Broeren et al., 2011; Kadkhoda et al., 2011; Le et al., 2016; Marschal et al., 2012; Pieretti et al., 2010).

The aim of this study was to investigate whether the automated flow cytometer parameters used for screening urine to rule out suspected UTI in current clinical practice correlate with the cultural isolation of the

*Abbreviations:* FCA, flow cytometry analyzer

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most significant bacterial urine pathogens.

## 2. Materials and methods

A retrospective study was conducted with urine samples assessed at the Microbiology Laboratory between June and September of 2015. Urines were collected from primary health care, outpatients, emergency and hospitalized patients. Samples from children were collected using pediatric bags and samples from adults were obtained by clean-catch midstream technique, using sterile wide-rim containers (approximately 10 ml of sample). 1.2 ml of urines samples were assessed with the Sysmex UF-1000i analyzer in the same day of arrival at Laboratory. Only those that met the criteria for culture (> 25 WBC/μL or > 385 bacteria/μL) according to the manufacturer's recommendations were subjected to a quantitative aerobic culture on chromogenic agar plates (CPS agar; bioMérieux, Marcy- l'Etoile, France) and incubated at 37 °C for 16–24 h. Culture criteria and satisfactory accuracy were confirmed prior to the study using a preliminary set of urine samples (data not shown). Samples were excluded from FCA analysis if excessive turbidity or blood was noted on visual inspection. Culture results were reported semi-quantitatively and classified in four categories as follows: negative, < $10^4$ CFU/mL, $10^4$–$10^5$ CFU/mL, > $10^5$ CFU/mL. Urine samples were defined as contaminated if more than two different pathogen species were detected in cultures. The diversity of microorganisms in contaminated urines was not documented. The microorganisms were identified by morphology and pigment in the CPS agar or using MALDI-TOF methodology (Vitek MS©, bioMérieux, Marcy-l'Etoile, France).

Patient demographics (age and gender), source of the urine sample, culture outcomes (CFU/mL) and flow cytometry parameters such as RBC, WBC and EC values were recorded and evaluated.

Data were analyzed using NCSS10 software 2015 for descriptive analysis. Dunn tests (R1.3.2 software) were conducted for comparisons between groups. Differences between groups were analyzed using the Kruskal-Wallis test. In the event of significant differences, multiple Dunn test comparisons were performed, adjusting the *p*-value by the Benjamini-Hochberg method.

The diagnostic performance with regard to the FCA variables bacteriuria and leukocyturia detected with the Sysmex UF-1000i as predictors of urine culture results was evaluated using Receiver Operating Characteristic (ROC) curves.

The BACT-morph software of Sysmex UF-1000i discriminates bacterial morphology and classifies bacteria as rods or cocci/mixed. Briefly, a fluorescent dye of nucleic acids added to the urine sample is detected in the bacteria channel. The forward-scattered fluorescence discriminates particle size while the side-scattered fluorescence indicates particle surface and complexity. Subsequently, the Sysmex UF-1000i analyzer classifies bacteria according to their fluorescence emission as rods or cocci/mixed. The performance of Sysmex UF-1000i to identify the presence of an infection caused by bacilli was evaluated in terms of sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV).

## 3. Results

A total of 17,483 urine samples were assessed with the Sysmex UF-1000i flow cytometer analyzer. Of these, 9057 (51.8%) met the criteria for culture examination. The reduction in urine cultures resulting from use of the flow cytometry screening was 48.2%. The median age of the patients was 47 years (P25-P75: 33.0–73.0) and most of them were women [85.3% (95% confidence interval: 84.6–86.0)]. Most samples originated in primary health care [77.7% (76.8–78.5)], and fewer from outpatients [15.7% (15.0–16.5)], emergency [4.1% (3.7–4.5)] and hospitalized patients [2.6% (2.3–2.9)]. In urine culture assessments we found 3725 [41.1%; (40.1–42.2)] negative cultures, 2146 [23.7% (22.8–24.6)] contaminated urines and 3186 [35.2% (34.2–36.2)] urines with bacterial growth. The most common pathogens were *Escherichia coli* [60.3% (58.6–62.0)], *Klebsiella spp.* [14.9% (13.7–16.1)] and *Enterococcus spp.* [12.0% (10.9–13.3)]. Demographic data, urine sample characteristics and culture results are shown in Table 1.

**Table 1**
Demographic characteristics and urine culture data.

| *N* = 9057 | | | n | % | (95%CI) |
|---|---|---|---|---|---|
| Patients | Age (years)[a] | | | 47 | (33.0–73.0) |
| | Gender | women | 7726 | 85.3 | (84.6–86.0) |
| | | men | 1331 | 14.7 | (14.0–15.4) |
| Urine sources | Primary health care | | 7034 | 77.7 | (76.8–78.5) |
| | Outpatients | | 1424 | 15.7 | (15.0–16.5) |
| | Emergency | | 368 | 4.1 | (3.7–4.5) |
| | Hospitalized patients | | 231 | 2.6 | (2.3–2.9) |
| Culture results | Negative | | 3725 | 41.1 | (1–42.2) |
| | Contaminated | | 2146 | 23.7 | (22.8–24.6) |
| | Bacterial growth | | 3186 | 35.2 | (34.2–36.2) |
| Urine pathogens | *Escherichia coli* | | 1922 | 60.3 | (58.6–62.0) |
| | *Klebsiella spp.* | | 473 | 14.9 | (13.7–16.1) |
| | *Enterococcus spp.* | | 381 | 12.0 | (10.9–13.3) |
| | *Proteus spp.* | | 132 | 4.1 | (3.5–4.9) |
| | Other gram-negative bacilli | | 178 | 5.6 | (4.8–6.4) |
| | Other microorganisms | | 100 | 3.1 | (2.6–3.8) |

95% confidence interval of relative frequency (95%CI).
[a] Median and interquartile range (IQR).

Significant differences ($p < 0.05$) in WBC, RBC, EC and bacteria counts were observed in certain culture results (Table 2 and Table 3). Negative urine cultures showed significant ($p < 0.05$) differences for all forming element counts in comparison with all other groups (Table 3). In particular, they yielded WBC counts (28.9 cells/μL) lower than *E. coli, Klebsiella* spp. and *Proteus spp.* (55.9, 55.1 and 54.4 cells/μL, respectively), but higher than *Enterococcus spp*. (18.9 cells/μL) and contaminated urine (15.6 cells/μL), as well as higher EC counts, with the exception of *Enterococcus spp*. (p = a) and *Proteus* spp. Cultures with *E. coli* and *Klebsiella* spp. growth did not show significantly different WBC, RBC, EC or bacteria counts. *E. coli* and *Proteus* spp. showed significant ($p < 0.001$) differences only in EC counts (4.6 cells/μL versus 7.4 cells/μL, respectively). *E. coli* and *Enterococcus* spp. showed significantly different values for all counts, with higher WBC counts but lower RBC and EC counts in *E.coli* cultures (12.6 cells/μL and 4.6 cells/μL versus 14.0 cells/μL and 4.2 cells/μL, respectively). Contaminated urine samples had significantly ($p < 0.001$) lower WBC counts than those with *E. coli*, *Klebsiella* spp. and *Proteus spp.*, but no differences ($p = 0.248$) were found with *Enterococcus spp.* (15.6 cells/μL and 18.9 cells/μL, respectively). No differences in EC count were observed between contaminated urine samples and samples with *Enterococcus spp*. ($p = 0.091$) or *Proteus spp*. ($p = 0.251$).

Performance in the diagnosis of gram-negative bacilli with Sysmex UF-1000i using the BACT-morph software showed a sensitivity of 81.4% (79.8–86.9), specificity of 48.1% (43.4–52.8), positive predictive value of 90.4% (89.1–91.5) and negative predictive value of 30% (26.7–33.6).

In the total sample analysis, the diagnostic performance of bacteriuria and leukocyturia as predictors of urine culture results yielded an area under the curve (AUC) value of 0.782 (0.772–0.792) for bacteria count and 0.605 (0.593–0.618) for WBC count (Fig. 1A). Excluding contaminated samples, the AUC increased to 0.844 (0.834–0.853) for bacteria, but decreased to 0.583 (0.570–0.597) for WBC (Fig. 1B). When the analysis sample was restricted to positive cultures, samples with over $10^5$ CFU/mL of bacteria (Fig. 1C), the AUC values were 0.798 (0.788–0.808) and 0.607 (0.594–0.619) for bacteria and WBC, respectively.

## 4. Discussion

As demonstrated by several other studies, the screening of urine