Performance assessment of urine flow cytometry (UFC) to screen urines to reflex to culture in immunocompetent and immunosuppressed hosts

Aleksandra Stefanovic,1,2 Diane Roscoe,1,2,* Romali Ranasinghe,3 Titus Wong,1,2 Elizabeth Bryce,1,2 Charlene Porter,1 Adelina Lim,4 Jennifer Grant,1,2 Karen Ng4 and Morris Pudek4

Abstract

Purpose. Urine flow cytometry (UFC) is an automated method to quantify bacterial and white blood cell (WBC) counts. We aimed to determine whether a threshold for these parameters can be set to use UFC as a sensitive screen to predict which urine samples will subsequently grow in culture.

Methodology. Urines submitted to our microbiology laboratory at a tertiary care centre from 22 July 2015–17 February 2016 underwent UFC (Sysmex UF-1000i) analysis, regular urinalysis and urine culture. Positive urine cultures were defined as growth $\geq 10^5$ c.f.u. ml$^{-1}$ of organisms associated with urinary tract infections. The correlation of UFC bacterial and WBC counts with urine culture was assessed using receiver operating characteristics curves. The sensitivity (SN), specificity (SP), negative predictive values (NPVs), positive predictive values (PPVs) and false negative rate (FNR) were calculated at various thresholds in immunocompetent and immunosuppressed patients.

Results. A total of 15 046 urine specimens were submitted, of which 14 908 were analysable in the study. The average time to UFC result from receipt in the laboratory was 0.76 h ($\pm$ 1.04 h). The test performance at a set threshold of UFC bacteria $\geq 20$ or WBC $> 5$ was: SN=96.0 %, SP=39.2 %, PPV=47.0 %, NPV=94.5 % and FNR=4.0 %. This threshold eliminates 26 % of urine cultures. Immunosuppressed hosts had a lower sensitivity of 90.6 % and a higher FNR of 9.4 %.

Conclusions. UFC is a rapid and sensitive method to screen out urine samples that will subsequently be negative and to reflex urines to culture that will subsequently grow. UFC results are available within 1 h from receipt and enable the elimination of culture when the set threshold is not met.

INTRODUCTION

Urinary tract infections (UTIs) are common infections, and urines are among the most tested clinical samples in the microbiology laboratory [1, 2]. However, approximately 60–80 % of urines submitted for culture are negative for bacterial growth [3]. To support the diagnosis of suspected UTIs, urine macroscopy (dipstick) for leukocyte esterase (LE) and nitrates is often used. Interpreted alone, LE and nitrites have a low and wide range of sensitivities (SN): 48–86 % and 45–60 %, respectively [4]. When both nitrates and leukocyte esterase are negative, SN increases to 68–88 %, suggesting that this combination could be useful for excluding UTI [4, 5], but is still not very effective at the lower SN. Urine microscopic examination for bacteriuria and pyuria is a more accurate method for urine screening (SN $\geq 94 %$, SP $\geq 70 %$), but it is more labour-intensive, requires technical expertise and has a longer turnaround time [6–9].

Urine culture is the gold standard for microbiological diagnosis of UTI, but reports may require 24–48 h to finalize, increasing the possibility of unnecessary antibiotic
prescribing [10]. Too often, clinicians forgo urinalysis and only request urine culture, missing key diagnostic information that can be obtained from a positive urine dipstick and which can be predictive of a positive urine culture in the presence of nonspecific urinary symptoms [9–11]. Although some laboratories have introduced the urine dipstick as a screening method for urine culture, there is no standardized approach to urine screening [12–16].

There is need for an accurate, rapid and efficient screening method for urines submitted for culture, to assist clinicians and reduce work-up of urines that ultimately test negative for growth. Urine flow cytometry (UFC) is an automated method that is capable of rapidly detecting and quantitating white blood cells (WBCs), bacteria and yeast, among other parameters. The aim of our study was to determine the diagnostic accuracy of UFC as a screening method for urine cultures and to establish a threshold with an acceptable sensitivity of ≥95 %, below which urine samples are likely to be negative for bacterial growth in culture. [17]. If an acceptable threshold using UFC parameters can be determined, it is our intention to reflex urine specimens to culture when the threshold is met, and eliminate specimens from culture if the threshold is not met.

METHODS
Study setting
Our institution is a 955-bed academic provincial referral hospital offering outpatient, tertiary and specialized services. Specimens submitted for urine culture over the period of 22 July 2015 to 17 February 2016 were included in the study.

Urinalysis
All urines underwent automated routine urinalysis coupled with UFC and urine culture. UFC was performed using the Sysmex UF-1000i (TOA Medical Electronics, Kobe, Japan), with the capability to evaluate 80–100 urine specimens per hour. The UFC system combines forward light scatter properties with fluorescent stain properties for laser-based fluorescent flow cytometry and has the ability to differentiate various formed urine elements, such as red blood cells (RBCs), WBCs, bacteria, yeast, casts and crystals. The urine sample is treated with fluorescent dyes (proprietary) to stain intracellular nuclear material contained within bacteria and other particles. The particles are transported to a flow cell and irradiated by a semiconducting laser (λ=635 nm). The combination of side scatter, forward scatter and fluorescent intensity are used to discriminate various formed elements using an adaptive cluster analysis system (ACAS). The system has been designed to maximize the ability to count bacteria, with a lower limit of detection of 5 bacteria per µl, and an analytical range of 5–10 000 bacteria per µl for a coefficient of variation (precision) reported to be 10 % or less (manufacturer’s product insert). Based on the literature for the UFC Sysmex, the average coefficient of variation for bacterial counts is 10 % and there is 95 % agreement with manual counts for WBCs [18].

The stability of specimens and preanalytical variables were controlled with our protocol for acceptable specimens. To be accepted into the study, the urine sample had to have a minimum volume of 2 ml, have been collected in standard containers and have been received in the laboratory within 2 h of collection or refrigerated at 4 °C.

Microbiology urine culture
Urine was inoculated onto a 5 % sheep’s blood agar plate (BAP) and a MacConkey plate (MAC) using a 0.001 ml quantitative inoculation loop. After overnight incubation at 35 °C in ambient air, the colony count (colony-forming units, c.f.u.) was measured semi-quantitatively (<10⁴, 10⁴–10⁵, >10⁵ c.f.u. ml⁻¹). Invasive urine specimens (collected by tube or drain directly into the kidney or urinary tract) were inoculated onto BAP and MAC using a 0.01 ml inoculation loop to enable the detection of c.f.u. of as low as 10² c.f.u. ml⁻¹. Pinpoint growth plates were incubated for 48 h. Colony identification was performed using MALDI-TOF Biotype 3.1 (Bruker Daltonics, Bremen, Germany) and antibiotic susceptibility testing was performed using the Phoenix Automated Microbiology System (BD Biosciences, Sparks, MD, USA). Counts of less than 10 000 c.f.u. ml⁻¹ (<10⁴ c.f.u. ml⁻¹) were defined as no growth or no significant growth [1]. The growth of ≥3 organisms with none predominating was reported as growth of mixed organisms, representing possible contamination The result of mixed growth was used for both mixed normal microbiota and for mixed Gram-negative bacilli with or without normal microbiota. Organisms that are usually associated with infection in the urinary tract were considered to be pathogens, while organisms that are typical commensals of the skin and perineum were considered to be non-pathogens.

Data collection
Data collection included patient demographics, ordering location, type of urine specimen (midstream, first void or catheter), times of urine collection and receipt in the laboratory, time of result, urine leukocyte esterase and nitrate, UFC parameters (bacteria, WBC, yeast and haemoglobin) and the urine culture results.

Subset analysis
The performance of UFC in immunosuppressed versus immunocompetent patient populations was analysed specifically using the unit location of the submitted specimens as a surrogate for immune status. The immunosuppressed group was defined as having specimens collected from specific wards or clinics known to exclusively admit or assess immunosuppressed hosts (the bone marrow transplant inpatient unit and outpatient clinic, the liver transplant inpatient unit and the solid organ transplant outpatient clinics). The immunocompetent group was defined as having specimens collected from wards typically admitting immunosuppressed patients, such as medicine and surgery as well as outpatient clinics. Units such as ED and ICU with mixed immunocompetent and immunosuppressed populations were excluded from the subset analysis.
Statistical analysis

Correlation of UFC bacteria and UFC WBC with urine culture results was performed using regression models. The performance of the modelled correlation was assessed visually using receiver operating characteristic (ROC) curves. Further granularity was obtained by calculating performance measures such as sensitivity (SN), specificity (SP), negative predictive value (NPV), positive predictive value (PPV) and false negative rate (FNR) at various thresholds of UFC bacteria and UFC WBC.

In order to assess the impact of other variables on the performance of the thresholds to predict urine culture positivity, age, gender, pathogen, immune status (determined by the surrogate marker of a known unit of order where immunosuppressed patients are located) and patient admission status (in- or outpatient) were included in the model in a stepwise fashion. A final multivariable model was based on the incremental increase in the model’s predictive ability. Further sub-group analysis of performance measures was performed, based on the significance of each variable at the univariate level. All statistical analysis was performed using R version 0.98.493.

RESULTS

A total of 15 046 urine samples were requested for urine culture during the study period. One hundred and thirty-eight (0.9 %) specimens were excluded, with culture not being performed, due to insufficient quantity or because they were not received in the laboratory. Of the 14 908 urine specimens available for analysis, 9549 (64 %) had no growth and 5359 (35.9 %) had growth ≥10^4 c.f.u. ml⁻¹ in culture. The majority of samples were from inpatient wards (7635, 51.2 %), the emergency department (4972, 33.4 %) and outpatient clinics (2301, 15.5 %), of which 70 % were from outpatient transplant clinics. Using the ordering unit of specimens submitted according to strict criteria as a surrogate for immune status, approximately 50 % of the samples could be designated as being from immunocompetent (40.3 %) or immunosuppressed populations (11.1 %); the remainder (48.6 %) could not be designated, as they were from ordering locations with mixed populations of both immune-competent and immune-suppressed patients. The majority of samples classified as coming from immunocompetent patients were from inpatients (5317, 88.5 %), most of whom were on the medical or surgical wards. Most samples classified as coming from immune-suppressed patients were from ‘high-risk’ outpatient areas (81.1 %), while 18.9 % were from inpatient units where only immunosuppressed patients were admitted, all of whom were transplant patients. The mean time to results of UFC from receipt of specimen in the laboratory was 0.76 h (+/-1.04 h). Additional patient and sample characteristics are detailed in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Age (mean years (sd))</th>
<th>Gender (female: male: other)</th>
<th>Specimen type (midstream: catheter: first void: other)</th>
<th>Admission status (inpatient: outpatient: high-risk outpatient*: emergency)</th>
<th>Immune status based on ordering location (immune-suppressed: immune-competent: could not determine)</th>
<th>Time to UFC result after receipt in the laboratory (mean hours (sd))</th>
<th>Urine culture results (growth (&gt;10^4 c.f.u.): no growth (&lt;10^4 c.f.u.))</th>
<th>Potential pathogens (in positive urine cultures) (pathogens: non-pathogens, pure: mixed growth†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62.29 (20.4)</td>
<td>7950 (53.3 %) / 6953 (46.6 %)</td>
<td>11 577 (77.7 %) / 2579 (17.3 %) / 703 (4.7 %) / 49 (0.3 %)</td>
<td>7635 (51.2 %) / 693 (4.7 %) / 1608 (10.8 %) / 4972 (33.4 %) / 1647 (11.1 %) / 6010 (40.3 %) / 7251 (48.6 %)</td>
<td>0.76 (1.04)</td>
<td>5359 (35.9 %) / 9549 (64 %)</td>
<td>3431 (64.0 %) / 217 (4.0 %) / 1711 (31.9 %)</td>
<td></td>
</tr>
</tbody>
</table>

*High-risk outpatient includes non-admitted patients whose samples were submitted from clinics with only immunosuppressed patients.
†A result of mixed growth is used when there was growth of ≥3 organisms with none predominating. This designation was used for both mixed normal microbiota and for mixed Gram-negative bacilli with or without normal microbiota.
Of the 5359 culture-positive samples, 3431 (64.0 %) grew potential pathogens causing urinary tract infections, 217 (4.0 %) grew non-pathogens that are normally part of the skin and perineal microbiota and 1711 (31.9 %) grew mixed organisms, and thus were uninterpretable. For the specimens that grew potential pathogens, *Escherichia coli* (43.4 %), *Klebsiella pneumoniae* (11.2 %) and *Enterococcus faecalis* (10.6 %) were the most common organisms. For the specimens with non-pathogens, the most common organisms were coagulase-negative staphylococci (67.7 %), while a few grew *Corynebacterium* species, lactobacilli and viridans group streptococci.

**Univariate models and ROC analysis**

In univariate models of urine culture positivity, both UFC bacteria and UFC WBC were significant predictors of a positive urine culture. In Fig. 1, ROC curves show how closely each UFC measure approximates urine culture results at different discrimination thresholds. Fig. 2 shows the ROC curve for UFC bacteria and/or WBC, used in combination to predict urine culture growth. The area under the curve (AUC) for UFC bacteria alone and UFC WBC alone were 0.86 and 0.78, respectively, while for the combination of UFC bacteria and/or WBC the AUC was 0.86.

**Multivariable analysis**

Bivariate models of the demographic and sample characteristics indicated that the odds of a positive culture decreased by about half in males compared to females (OR=0.48, *P*<0.001). Age was not associated with a positive urine culture. When examined by patient location, inpatient, outpatient and transplant outpatient samples all had significantly lower odds of a positive culture compared to samples collected from emergency department patients (OR=0.44, 0.83 and 0.49, respectively, *P*<0.001). Immunocompetent patients were associated with more than double the odds of a positive urine culture compared to immunosuppressed patients (OR=2.12, *P*<0.001). Stepwise addition of the key variables identified in the bivariate analysis resulted in increased deviance and Akaike information scores, while lowering the log-likelihood ratio compared to the original model. This left the UFC bacterial count as the only variable closely associated with positive urine culture.

**Thresholds and performance**

Upon investigating the performance of UFC bacterial and WBC counts at various thresholds, at UFC bacterial counts ≥20 we were able to achieve SN=94.3 %, SP=45.6 %, PPV=49.3 %, NPV=93.4 % and FNR=5.7 %. Although ROC and multivariable analysis indicate that UFC bacterial count is the closest predictor of a positive urine culture, when we combined the performance measures at UFC bacterial threshold of ≥20 and/or UFC WBC >5, the desired sensitivity for a screening test of ≥95 % was achieved (Table 2).
Table 2. Performance of threshold UFC levels (either bacteria $\geq 20$ OR/ WBC $>5$) to predict subsequent growth of urine culture, stratified by patient immune status as determined by ward location

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>FNR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>96.0 (95.7–96.3)</td>
<td>39.2 (38.4–40.0)</td>
<td>47.0 (46.2–47.8)</td>
<td>94.5 (94.1–94.9)</td>
<td>4.0 (3.7–4.3)</td>
</tr>
<tr>
<td>Immunosuppressed</td>
<td>90.6 (90.1–91.1)</td>
<td>46.6 (45.8–47.4)</td>
<td>30.5 (29.8–31.2)</td>
<td>95.0 (94.6–95.3)</td>
<td>9.4 (8.9–9.9)</td>
</tr>
<tr>
<td>Immunocompetent</td>
<td>95.9 (95.6–96.2)</td>
<td>40.1 (39.3–40.9)</td>
<td>46.8 (46.0–47.6)</td>
<td>94.7 (94.3–95.1)</td>
<td>4.1 (3.8–4.4)</td>
</tr>
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Sub-group analysis of UFC performance based on immune status

Using strict criteria for unit of order as a surrogate for immune status, 1647 patients were considered to be immunosuppressed. Table 3 summarizes the characteristics for these patients. Most samples classified as being from immune-suppressed patients were from ‘high-risk’ outpatient areas (81.1%) while 18.9% were from inpatient units where only immunosuppressed patients were admitted, all of whom were transplant patients. In the immunosuppressed group only 20.6% of urines were positive in culture. A sub-group analysis of the performance of the set threshold shows differences in the performance measures between immunosuppressed and immunocompetent patients (Table 2). UFC performance in immunosuppressed patients demonstrated SN of 90.6%, SP of 46.6%, PPV of 30.5%, NPV of 95.0% and FNR of 9.4%. The SN was statistically significantly lower and the FNR significantly higher in the immunosuppressed group compared to the immunocompetent population (SN=90.6% vs SN=95.9%; FNR 9.04% vs 4.0%; Z=-4.91, P<0.01).

The median WBC count in immunocompetent patients was higher than that in immunosuppressed patients [3.0, inter-quartile range (IQR) 1.0–23.0 vs 2.0, IQR 1.0–7.0; P<0.005]. The median bacterial count was also significantly higher among immunocompetent compared to immunosuppressed patients (54.0, IQR 13.0–480.8 vs 24.0, IQR 9.0–83.5; P<0.005) (Table 4). The proportion of Gram-positives in immunosuppressed patients was statistically significantly higher than in immunocompetent patients (23.3 vs 18.8%, P<0.05).

Furthermore, when comparing samples growing Gram-positive bacteria against samples growing Gram-negative bacteria, the FNR was significantly higher (6.8%, 95% CI 5.3–8.7 vs 1.0%, 95% CI 0.5–1.9; P<0.005) and the SN was significantly lower (93.2 vs 99%; P<0.005) (Table 5). Although the proportion of samples that grew yeast was not significantly different in immunosuppressed patients versus immunocompetent patients, the UFC sensitivity in samples growing yeast was significantly poorer than in samples with Gram-negative bacteria (93.2%, 95% CI 87.9–96.4 vs 99%, 95% CI 98.4–99.4; P<0.05).

Table 3. Patient characteristics for the immunosuppressed group as determined by ordering location (n=1647)

| Characteristic                        | Mean years (sd) | Gender Number (%): Male (58.9%), Female (41.1%) | Patient location Number (%): Kidney transplant clinic (58.5%), Bone marrow transplant (BMT) inpatient ward (18.9%), BMT outpatient clinic (11.8%), Solid organ transplant inpatient unit (6.7%), Lung transplant clinic (1.8%), Pancreas/kidney transplant clinic (1.0%), Pancreatic islet transplant clinic (0.9%), Pre-transplant Assessment Clinic (0.3%) | Time to UFC result after receipt in the laboratory Mean hours (sd) 0.74 (0.0) | Urine culture results Growth ($\geq 10^4$ c.f.u. ml$^{-1}$) Number (%) 339 (20.6%), No growth (<$10^4$ c.f.u. ml$^{-1}$) Number (%) 1308 (79.4%) | Potential pathogens (in positive urine cultures) Pathogens Number (%) 193 (56.9%), Non-pathogens, pure Number (%) 28 (8.3%), Mixed growth* Number (%) 118 (34.8%) |

*A result of mixed growth is used when there was growth of $\geq 3$ organisms with none predominating. This designation was used for both mixed normal microbiota and for mixed Gram-negative bacilli with or without normal microbiota.
The average number of samples per patient was higher among the immunosuppressed patients (3.3 samples versus 1.4 samples in immunocompetent patients; t=12.63, P<0.005) (Table 4). At the unit level, only in the kidney transplant clinic did the mean number of samples per patient significantly exceed the average for immunocompetent samples (t=4.7, P<0.05) and all samples (t=11.3, P<0.05). The FNR among single-sample patients was not significantly different from that among patients with multiple samples.

Table 6 describes the organism distribution among the 32 UFC false negative (FN) samples. Antibiotic prescription data were available for 26/32 patients, and of these only 2 received antibiotic treatment. In one case a BMT patient with urine culture growing 6 x 10^6 c.f.u. ml^{-1} of mixed organisms was treated with ciprofloxacin, and in the other a liver transplant patient with urine catheter culture growing > 10^5 c.f.u. ml^{-1} of yeast received meropenem and micafungin.

**UFC as a screen to reflex to urine culture**

Using UFC parameters to maximize sensitivity, 10 945 urine samples met the threshold of bacteria ≥20 and WBC >5, and would have been reflexed to culture. The remaining 3963 (26.6% of analysable specimens) did not meet the threshold and would not have been reflexed to culture.

**DISCUSSION**

Our study of 14 908 urine samples is the largest study to date evaluating the UFC Sysmex 1000i as a screening method for urine cultures. UFC allowed for the rapid screening of urines within 1 h of receipt in the laboratory, with minimal hands-on time and technical expertise. The results of univariate and bivariate regression models confirmed that both UFC bacterial and UFC WBC counts were significant predictors of positive urine cultures. However, when tested in multivariate models, only UFC bacterial counts were related to positive urine cultures. We were able to achieve the desired sensitivity ≥95% when combining the parameters of traditional microscopy with UFC bacteria counts ≥20 and/or UFC WBC >5 [17]. Therefore, for urines to undergo culture, either of these thresholds must be met, otherwise they will not be cultured. Furthermore, with a low FNR of 4.0% we would miss very few urines that would be positive on culture, while being able to eliminate 26% of urines from undergoing culture.

A number of studies have been published evaluating the Sysmex UF 1000i flow cytometer as a screening method for urine cultures [17–26]. The studies have included different patient populations, used different criteria to determine urine culture positivity, aimed at different desirable performance characteristics (SN and NPV) and arrived at different conclusions as to the ideal cut-off values for UFC bacterial and/or UFC WBC counts. A systematic review and meta-analysis of the published studies by Shang et al. included studies of variable design, using different analysers (UF100 or UF1000i), bacterial and WBC cut-off values, and reference standards for positive urine cultures, making it a challenging comparison [27]. Nevertheless, they estimated a pooled sensitivity of 92% for UFC bacteria and one of 87% for UFC WBC, concluding that UFC may be a promising screening tool for urine cultures.

A similar study to ours by Kadkhoda et al. evaluated 2496 inpatient urines, using a similar microbiological reference standard for positive urine culture, and found that UFC bacterial count ≥20 resulted in a sensitivity of 93% and allowed

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity (95% CI)</th>
<th>FN* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positives, n=921</td>
<td>93.2 (91.3–94.7)</td>
<td>6.8 (5.3–8.7)</td>
</tr>
<tr>
<td>Gram-negatives, n=2192</td>
<td>99.0 (98.4–99.4)</td>
<td>1.0 (0.5–1.9)</td>
</tr>
<tr>
<td>Yeast, n=163</td>
<td>93.2 (87.9–96.4)</td>
<td>6.8 (3.6–12.1)</td>
</tr>
<tr>
<td>All samples, n=14 908</td>
<td>96.0 (95.7–96.3)</td>
<td>4.0 (3.7–4.3)</td>
</tr>
</tbody>
</table>

*FN, False negative samples are those that grew in culture but which did not meet the UFC threshold criteria.
the rejection of 35% of urines from culture [23]. Broeren et al. evaluated 1577 inpatient and outpatient urines, and determined that a UFC bacterial cut-off of 39 yielded a sensitivity of 95% [22]. These differences in performance likely reflect pre-test probability and patient settings. For instance a meta-analysis found that the pre-test probability of UTI was higher in an outpatient setting than in hospital studies because outpatient providers use the dipstick to diagnose a UTI based on clinical signs and symptoms, whereas in hospitals the urine dipstick test may be used to screen patients in order to exclude the presence of infection [4].

While *E. coli* is the most predominant pathogen in both inpatient and outpatient settings, the organism epidemiology found in our study is more reflective of hospital pathogens. In our study, the majority of organisms (64%) isolated were considered to have pathogenic potential. There was also a large proportion of mixed cultures (31.9%), which could reflect issues with specimen collection and prolonged transport, indicating a need to revisit the pre-analytical phase of culturing urines. Timely transport to the laboratory with fast turn-around time of UFC to negative result would allow clinicians to rule out UTI as a source of infection rapidly and pursue other clinical diagnoses. As 3963 (26.6%) of urines did not meet the threshold set in our study, they would not have undergone culture, leading to reduced microbiology workload.

To date, no data have been published on the performance of UFC in the immunosuppressed population. A subset analysis of 1647 urines from this population revealed a lower positivity rate of 20.6% as well as a lower SN of 90.6% and PPV of 30.5%, and a higher NPV of 9.4% at the set threshold. Antibiotic prophylaxis prescribed to transplant patients, typically with trimethoprim/sulfamethoxazole (TMP/SMX), could be partly responsible for the lower urine positivity rate and the lower PPV. Although a larger number of samples was submitted per patient in the immunosuppressed group (especially kidney transplant recipients) this did not account for the higher FNR. Recent studies have shown that between 87–92% of urines cultured in kidney transplant clinics represent asymptomatic bacteriuria (AB), and that systematic screening and treatment for AB beyond 2 months post-kidney transplant had no clear benefit [28, 29]. Subsequently, routine screening of kidney transplant patients at our institution has been discontinued, which would likely have an effect on number of urines submitted, but not on the FNR.

For immunosuppressed patients, the median UFC bacterial and WBC counts were lower. The lower WBC counts may be explained by the presence of neutropenia and decreased ability to mount an immune response in some of the patients. The difference in average bacterial counts may be due to antibiotic prophylaxis and different organism distribution, with a higher proportion of Gram-positive organisms at 23.3% in immunosuppressed patients. For all of the patients, UFC performance with urines with Gram-positive organisms resulted in a lower sensitivity of 93.2% and a higher FNR of 6.8% compared to Gram-negative organisms. This suggests that UFC is less sensitive for the Gram-positive organisms, which are found at a higher proportion in immunosuppressed patients, which may contribute to the higher FNR. Kadkhoda et al. also found a difference in UFC sensitivity between Gram-positive and Gram-negative organisms, with an overall sensitivity of 99.2 and 85% for Gram-negative and Gram-positive pathogens, respectively [23]. Researchers have speculated that the Gram-positive organisms tend to form aggregates, leading to lower counts by cytometry [19]. The difference in organism distribution in immunosuppressed hosts could be due to the prophylaxis regimen with TMP/SMX potentially selecting for organisms with resistance to this agent. Interestingly, we also observed lower SN for yeast cells at 93.2%, which has previously been reported for *C. albicans* at >10⁶ c.f.u. ml⁻¹ being interpreted as RBC by UFC, therefore lowering the sensitivity of detection [21]. Despite slightly lower sensitivity in the immunosuppressed population we consider the algorithm to be acceptable in this group, since physicians are aware that any urine will be cultured at their request based on their clinical assessment and regardless of UFC results.

In order to explore the higher FNR further, we looked at the false negative specimens in more detail, as per Table 6. The relative split between Gram-positive and Gram-negative organisms was 11 to 4, while the rest of urines grew yeast and mixed organisms. The majority of the isolated organisms were either intrinsically resistant or tested resistant to TMP/SMX (14/15, 93.3%). Lastly, of the patients in the FN group for whom antibiotic data were available, only two received antibiotic treatment: one for urine culture growing mixed organisms resulted in a lower sensitivity of 93.2% and a higher FNR of 6.8% compared to Gram-negative organisms. Kadkhoda et al. also found a difference in UFC sensitivity between Gram-positive and Gram-negative organisms, with an overall sensitivity of 99.2 and 85% for Gram-negative and Gram-positive pathogens, respectively [23]. Researchers have speculated that the Gram-positive organisms tend to form aggregates, leading to lower counts by cytometry [19]. The difference in organism distribution in immunosuppressed hosts could be due to the prophylaxis regimen with TMP/SMX potentially selecting for organisms with resistance to this agent. Interestingly, we also observed lower SN for yeast cells at 93.2%, which has previously been reported for *C. albicans* at >10⁶ c.f.u. ml⁻¹ being interpreted as RBC by UFC, therefore lowering the sensitivity of detection [21]. Despite slightly lower sensitivity in the immunosuppressed population we consider the algorithm to be acceptable in this group, since physicians are aware that any urine will be cultured at their request based on their clinical assessment and regardless of UFC results.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of specimens, n=32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>13</td>
</tr>
<tr>
<td>Vancomycin-resistant <em>Enterococcus faecium</em></td>
<td>4</td>
</tr>
<tr>
<td>Yeast</td>
<td>4</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>3</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococi</em></td>
<td>3</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Corynebacterium species</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas species</em></td>
<td>1</td>
</tr>
</tbody>
</table>

There are limitations to our study. This is a retrospective analysis of UFC performance compared to urine culture used as a diagnostic gold standard. However, as urine culture alone cannot distinguish between true UTI versus asymptomatic bacteriuria, the presence of symptoms is an...
important factor in making that distinction and was not available to us at the time of the study. This study was predominantly performed on inpatient population at a single centre and may not reflect other centres and outpatient populations. Our subset analysis relied on patient locations housing transplant populations as an indicator of immunosuppression, but patients admitted to medical and surgical units could have some level of immunosuppression that could not be accounted for.

In conclusion, UFC at a set threshold is an effective method to screen urine specimens that ultimately will be culture-negative. Screening urines prior to cultures ensures that all urines undergo a form of urinalysis, allows for rapidly available results for negative urines and decreases the workload in the microbiology laboratory. The decreased sensitivity of UFC observed in immunocompromized patients could be explained in part by the predominance of Gram-positive organisms, for which UFC appears to have lower sensitivity compared to Gram-negative organisms.

References