Ann Clin Microbiol Vol. 21, No. 4, December, 2018
https://doi.org/10.5145/ACM.2018.21.4.75
pISSN 2288-0585 · eISSN 2288-6850

Selection of Unnecessary Urine Culture Specimens Using Sysmex UF-5000 Urine Flow Cytometer

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Background: Urine culture is one of the most frequently requested tests in microbiology. Automated urine analyzers yield much infection-related information. The Sysmex UF-5000 analyzer (Sysmex, Japan) is a new flow cytometry urine analyzer capable of quantifying urinary particles, including bacteria, WBCs, and yeast-like cells (YLCs) and can provide a Gram stainability flag. In this work, we evaluated how many unnecessary urine cultures could be screened out using the UF-5000.
Methods: We compared the culture results of 126 urine samples among 453 requested urine cultures (from sources other than the Urology and Nephrology departments) with urinalysis results. Urine cultures were considered positive if bacterial or YLC growth was $\geq 10^4$ CFUs/mL.
Results: We used urinalysis cut-off values of 50/μL and 100/μL for bacteria and YLC, respectively. Forty eight of the 126 (38.1%, or 10.6% of 453 requested) cultures were below these cut-off values and did not contain any culture-positive samples.
Conclusion: Bacteria and YLC counts generated using the UF-5000 analyzer could be used to screen out negative cultures and reduce urine culture volume by ~10% without sacrificing detection of positive cultures. **(Ann Clin Microbiol 2018;21:75-79)**

Key Words: Sysmex UF-5000 flow cytometer, Unnecessary urine cultures, Urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) refers to the presence of microbial pathogens within the urinary tract. UTIs represent the most frequently occurring infectious diseases in hospitals and community populations [1]. Catheter-associated UTI is the most common nosocomial infection [2], and genitourinary infections are the second most common form of infection among non-institutionalized elderly [3]. The gold standard diagnostic method for UTI is urine culture, but it takes time and manpower. Rapid screening methods such as flow cytometry have been introduced to reduce the number of urine samples requiring cultures. The Sysmex UF-100 and UF-1000i units (Sysmex, Kobe, Japan) are automated urine flow cytometers that can detect particles, including leukocytes and bacteria, in urine, and some have compared urine bacterial and leukocyte counts with urine culture results with respect to the diagnosis of UTI [4-7]. Recently, the UF-1000i was updated to the Sysmex UF-5000 (Sysmex), which has better accuracy for bacterial quantification and stainability [8]. We considered this advantage might provide better performance for the screening out of negative urine samples. Accordingly, we investigated whether WBC, bacteria, and yeast-like cell (YLC) counts and Gram stainability flag results of the Sysmex UF-5000 could be used to screen out unnecessary urine cultures.

MATERIALS AND METHODS

1. Selection of samples

Four hundred and fifty three urine culture specimens were submitted to the microbiological laboratory at Pusan National University Yangsan Hospital (PNUYH) from 22nd, March through 1st, April, 2016. Of these samples only those with requests for urinalysis on day of sampling and subjected to uri-

Received 8 March, 2018, Revised 11 June, 2018, Accepted 22 June, 2018

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nalysis within one hour of commencement of urine culture were selected. Specimens submitted from Departments of Urology and Nephrology from PNUYH and PNUYH Children's Hospital were excluded, and thus, this study was conducted on selected 126 specimens.

2. Urine culture and urinalysis

Cultures were performed according to the standard protocol used at PNUYH. Briefly, clean-caught midstream urine was submitted to the laboratory within 2 hours of collection. One microliter of sample was inoculated onto 5% blood agar and MacConkey agar plates (both from Micromedia, Busan, Korea) and incubated in 37°C for 24 h to 48 h before reading. After incubation, colonies were counted, and identification and susceptibility testing were performed by using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). Urine cultures were considered positive if bacterial or yeast growth exceeded 10^4 CFU/mL. When ≥3 types of colonies without a dominant microorganism were cultured, the sample was considered to have been contaminated.

Urinalysis was performed by using the AUTION MAX AX-4030 strip analyzer (ARKRAY, Kyoto, Japan), and the Sysmex UF-5000 analyzer. Urine sediment interpretation was performed manually, but results were not used in this analysis.

3. Selection of unnecessary cultures

To distinguish unnecessary cultures from among the 126 urine cultures, we used four criteria; (i) 'negative (−)' or 'trace (+/−)' leukocyte esterase, and 'negative (−)' urine nitrite by urine reagent strip analysis, and an 'unclassified' flag in UF-5000 result, (ii) bacteria, (iii) YLCs, and (iv) WBC counts in UF-5000 result. 'Flag' means Gram stainability, and is expressed as Gram positive, Gram negative, Gram positive/negative, unclassified, or no flag. When the urine cultures were compared with the bacterial counts, only bacterial growth was considered, except yeast growth in urine. In the same manner, in comparison of the urine cultures with the YLC counts, only yeast growth was considered, except bacterial growth. However, for WBC counts, both bacterial and yeast growth were considered in urine cultures. Urine cultures were used as the reference standard, and the statistical analysis was performed using IBM SPSS version 21 (IBM, Armonk, NY, USA).

4. Ethics approval

Ethics approval for the study was obtained from the institutional review board (IRB) of Pusan National University Yangsan Hospital (05-2016-041).

RESULTS

Among the 126 urine samples, 46 isolates of microorganisms were grown from 41 samples (32.5%); 12 *Escherichia coli*, 7 *Enterococcus faecalis*, 5 *Enterococcus faecium*, and 22 other bacteria or yeasts (Table 1), which means that 85 (67.5%) samples were negative by culture.

When using the three parameters, leukocyte esterase, nitrite and flag, to select unnecessary culture requests, 69 of the 85

Table 1. Microorganisms and number isolated in urine cultures

Microorganism	No.
Enterococcus faecalis	7
Enterococcus faecium	5
Granulicatella adiacens	1
Staphylococcus epidermidis	2
Gram-positive nonsporeforming bacilli	2
Acinetobacter baumannii	2
Citrobacter freundii	1
Enterobacter cloacae	1
Escherichia coli	12
Klebsiella pneumoniae	3
Proteus mirabilis	2
Pseudomonas aeruginosa	2
Candida albicans	2
Candida glabrata	3
Candida tropicalis	1
Total	46

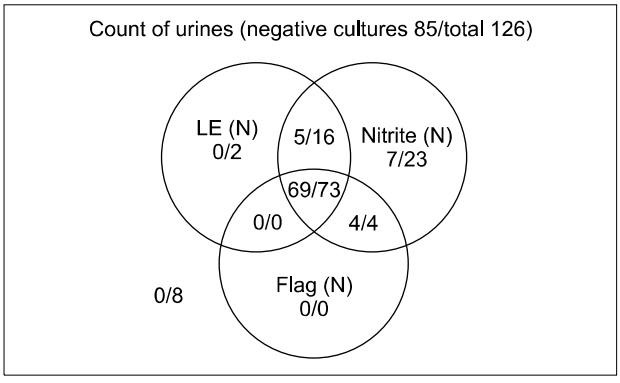

Fig. 1. Classification of culture negative urine samples using the three urinalysis parameters. 1) LE (N), negative (−) or trace (+/−) leukocyte esterase by urine reagent strip analysis; 2) nitrite (N), negative (−) nitrite by urine reagent strip analysis; and 3) Flag (N), no or unclassified flag by UF-5000 analysis. Abbreviations: LE, leukocyte esterase; N, negative.

(81.2%) culture negative samples were negative or trace for leukocyte esterase and negative for nitrite by urine reagent strip analysis, and 'no' or 'unclassified flag' by UF-5000 analysis (Fig. 1). However, using the same criteria, a significant portion of culture positive urines (4/41, 9.8%) were classified in the same way.

Distributions of bacteria, YLC, WBC counts and culture results are shown in Fig. 2. Mean bacteria, YLC and WBC counts were significantly lower in the culture negative group than in the culture positive group ($P<0.0001$). Receiver operating characteristic (ROC) curves for UF-5000 WBC, bacteria and YLC counts are shown in Fig. 3. Areas under the curves (AUCs) for the WBC, bacteria and YLC counts were 0.74 (95% CI=0.68-0.81), 0.90 (95% CI=0.86-0.94), and 0.92 (95% CI=0.82-1.00), respectively. As shown in Fig. 3, the cut-off values of bacteria and YLC were shifted to the right. This was because our aim was not to find optimal cutoffs based on considerations of sensitivity and specificity, but to find cutoffs that could be used to screen out culture negative samples. When a bacteria count of 50/μL was used the cut-off, 49 negative cultures (including one positive yeast culture) were below this level and these samples did not contain any culture positive samples. When a YLC count of 100/μL was used as the cut-off value, 114 negative urine yeast cultures (including 33 positive bacterial cultures) were below this level, and no positive yeast culture sample was included. For bacteria and YLC counts combined, 48 of the 85 (56.5%) negative urine cultures (38.1% of the 126 samples analyzed; 10.6% of the 453 requested cultures) were found to be

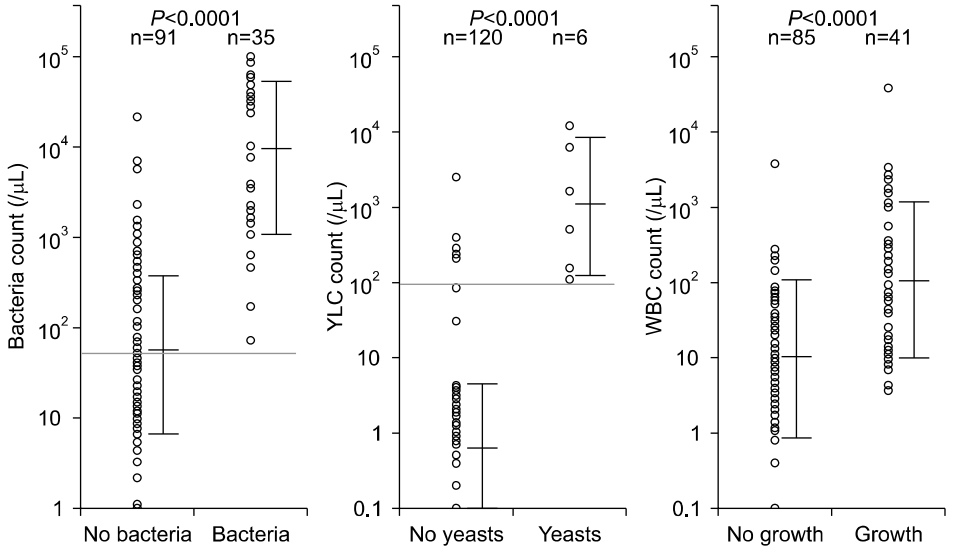

Fig. 2. Distribution of WBC, bacteria and YLC counts according to culture results. The UF-5000 analyzer produced a result of 10^5 for bacterial counts $>10^5$. A bacterial count of 0 is expressed as 1.0 and a YLC count of 0 as 0.1 (these values were minimum values in bacterial and YLC count data set, respectively) because the X-axis was transformed to a log scale. Abbreviations: YLC, yeast-like cell; WBC, white blood cell.

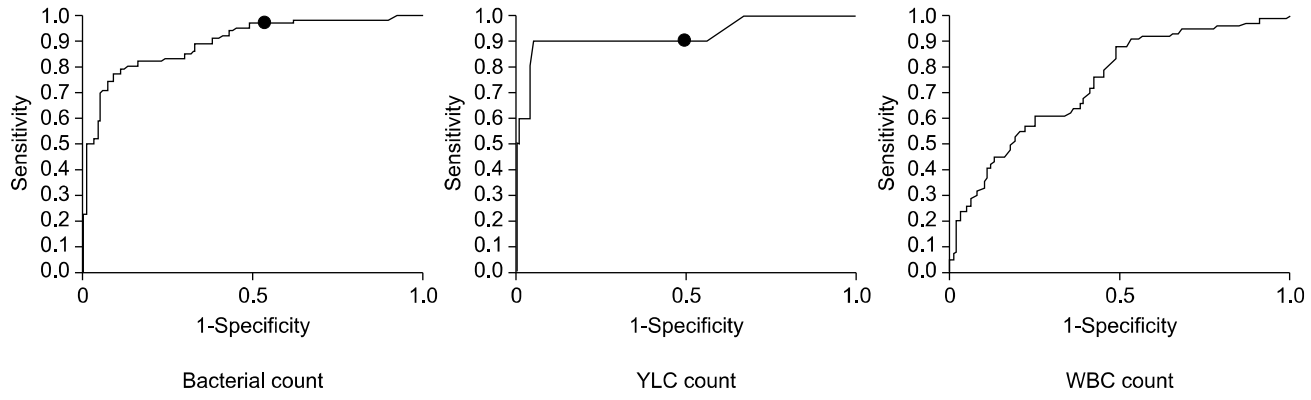

Fig. 3. Receiver operating characteristic (ROC) curves of WBC, bacteria and YLC counts versus urine culture results. The red marks in left and center graphs indicate the cut-off values for bacteria (50/μL) and YLCs (100/μL), respectively. Abbreviations: YLC, yeast-like cell; WBC, white blood cell.

below each cut-off value, and no culture positive urine sample was included. WBC count did not well differentiate culture positive and negative samples. More specifically, when a WBC count of 3/ μL was used as the cut-off value, only 22 negative cultures had a count below the cutoff.

DISCUSSION

Urinalysis is performed as a primary routine screening test for almost all in- and outpatient evaluations, and is one of the most frequently requested tests in hospital laboratories. Traditionally, urinalysis is performed using the urine reagent strip test and a manual urine sediment examination. However, recent advances made it possible to perform sediment analysis automatically. These analyzers use two analytical principles for urine sediment analysis. One type is based on image analysis, such as, the IRIS IQ200 analyzer (Iris Diagnostics, Chatsworth, CA, USA) [9], whereas the other is based on flow cytometry, such as, the Sysmex UF Series. These analyzers count many types of particles accurately in a very short time, and classify particles by shape, size and other characteristics, and thus, can predict urine culture results and possibly reduce unnecessary urine cultures by reducing unnecessary urine cultures without sacrificing positive urine cultures. The Korean healthcare insurance system is considering the adoption of diagnosis-related groups (DRGs) for reimbursement, instead of cost-based reimbursement, which means that reducing unnecessary laboratory tests is equivalent to cost savings. Actually, cost calculations on this topic have been previously described. Kim et al. [6] reported a cut-off value of 1,500/ μL for bacterial count using the Sysmex UF-100 analyzer could reduce urine cultures by ~40% without any false negative results. Broeren et al. [4] using a negative culture definition of $<10^4$ CFU/mL, determined a reduction in unnecessary urine cultures of 28% with 5% false negative results at a bacterial cutoff of 39 / μL using the UF-1000i, and De Rosa et al. [5] reported 1.2% false negative results with a culture reduction of 57.1% at a bacterial cutoff of 170 / μL and a WBC cutoff of 150 WBCs / μL for the same unit.

The definition used for a positive urine culture result obviously affects screening performance. Some authors consider a urine culture positive if it is $>10^3$ CFU/mL [6], whereas others consider a urine culture positive if it is $>10^5$ CFU/mL [10], and thus, sensitivity and specificity are dependent on the definition used even though at the same cut-off values. For example, when the definition of a negative culture value is changed from $<10^4$ to $<10^5$ CFU/mL, sensitivity increases from 82% to 96%, while specificity decreases from 83% to 78%, respectively, at the same UF-1000i cutoff value for bacteria (200 / μL) [4]. In general, $\geq 10^5$ CFU/mL is considered significant bacteriuria as most symptomatic patients usually have $\geq 10^5$ bacteria/mL in bladder [11]. However, colony counts used to define significant bacteriuria depend on clinical symptoms, age, gender, urine collection method, isolated species and other factors.

The present study was conducted to determine whether analyzer based testing could predict which urine samples would produce a negative culture result, and not to detect specimens with bacteriuria, which meant the identification of culture negative urines with no or minimal false negative results. This is why we selected $<10^4$ CFU/mL as screening criteria for culture negatives. Actually, $<10^3$ CFU/mL would have been more conservative, but only one of the 126 samples had a count between 10^3 and 10^4 CFU/mL (data not shown). A medical record review showed this male patient was treated for respiratory disease and his urine culture result was not clinically significant. So, we tentatively defined urine culture positivity as 10^4 CFU/mL in this study.

We also evaluated whether urine reagent strip analysis and UF-5000 results predicted urine culture results. When the two reagent strip parameters, leukocyte esterase and nitrite, and one UF-5000 parameter, Gram stainability flag were used, the number of negative cultures required was reduced by 81.2%, but ~ 10% of culture positive samples were excluded. However, using a bacteria count of <50/ μL and a YLCs count of 100/ μL as determined by the UF-5000 analyzer, 56.5% of negative cultures requested from departments other than Urology and Nephrology, were classified as unnecessary, and these samples did not contain any positive urine samples. The reason why we excluded samples from Urology/Nephrology Departments is that we assumed specimens from those departments were more likely to be specific disease-oriented and inappropriate to screen for unnecessary cultures. Different criteria should be evaluated for such samples. Ratio of urine cultures requested by Urology/ Nephrology departments and others are probably dependent on patient characteristics and hospital size. PNUYH is a 1200-bed tertiary-care general hospital, and 126 of 453 urine specimens were submitted for culture by departments other than Urology and Nephrology during the 11 day evaluation period. Based on the findings of the present study, we estimate that the described screening method would obviate the need to test 10.6% of total samples submitted for urine culture.

In conclusion, the bacteria and YLC count results obtained using the UF-5000 analyzer could be used to predict negative cultures, and reduce urine culture volumes by ~10% above without sacrificing positive urine cultures.

ACKNOWLEDGMENTS

This work was supported by the annual clinical research grant from Pusan National University Yangsan Hospital (2018).

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=국문초록=

Sysmex UF-5000 소변 유세포분석기를 이용한 요배양 불필요 검체의 선별

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송두열, 이현지, 조수연, 이선민, 장철훈

배경: 요배양검사는 요로감염 진단을 위한 표준검사로 가장 흔히 의뢰되는 미생물 배양 검사 중 하나이다. 소변 자동분석기는 감염과 관련된 많은 정보를 제공한다. 최근 개발된 Sysmex UF-5000 (Sysmex, Japan)은 유세포분석 방법에 의해 세균, 효모균, 백혈구 등의 입자를 정량적으로 측정하고, 그람 염색성 정보를 제공한다. 저자들은 UF-5000을 이용하여 불필요한 요배양검사를 얼마나 선별할 수 있는지 평가하였다.

방법: 요배양검사가 의뢰된 453 검체 중 비뇨기과/신장내과 의뢰 검체를 제외한 126 검체를 대상으로 요시험지봉검사와 UF-5000으로 검사를 시행하여 요배양검사 결과와 비교하였다. 소변 배양은 집락수가 10^4 CFU/mL 이상인 경우 양성으로 판정하였다.

결과: UF-5000의 세균 수 50/μL 이하, 효모양 세포 100/μL 이하를 기준으로 했을 때 분석 대상 요배양의 38.1% (48/126), 전체 요배양 453건의 10.6%를 불필요한 요배양검사로 선별해 낼 수 있었다.

결론: UF-5000에서 산출된 세균 및 효모양 세포의 수로 음성 배양 결과를 예측할 수 있으며 약 10%의 불필요한 배양검사를 줄일 수 있다. [Ann Clin Microbiol 2018;21:75-79]

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