RESEARCH ARTICLE

Rapid differentiation of cocci/mixed bacteria from rods in voided urine culture of women with uncomplicated urinary tract infections

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Objectives: To evaluate the ability of laser flow cytometry to predict cocci/mixed growth in the pre-analytical phase of urine specimens.

Methods: We retrospectively reviewed urine samples from women with uncomplicated urinary tract infections from urologic clinics for study. Urine analyses were performed with laser flow cytometry (UF1000i, Sysmex, Kobe, Japan) and then diagrams were generated (forward scatter vs. fluorescent light scatter). Each specimen (bacteria count >357 BACT/ μ L) was classified as either cocci bacteria or rods/mixed growth according to the diagrams. Standard urine cultures were performed, and the agreement between cultures and the UF1000i interpretations was analyzed with kappa statistics.

Results: Finally, 491 specimens met the criteria for analysis. Among the 376 specimens with single bacteria growth, there were 26 gram-positive cocci (13 *Streptococci* spp., 7 *Staphylococci* spp., 6 *Enterococci* spp.), 1 gram-positive rods (*Corynebacterium* spp.), and 349 gram-negative rods (273 *Escherichia coli*, 33 *Klebsiella* spp., 29 *Proteus* spp., 6 *Citrobacter* spp., 4 *Enterobacter* spp., 3 *Pseudomonas* spp., and 1 *Providencia* spp.). There were 115 specimens with two bacteria species or more that were regarded as mixed growth. Agreement of rods or cocci/mixed growth between the laser flow cytometry and urine cultures yielded a kappa value of 0.58. The positive and negative predictive rate of the UF1000i for cocci/mixed growth in voided urine culture was 81.8% and 84.7%, respectively.

Conclusions: Through laser flow cytometry, we can predict growth of cocci/mixed growth in the pre-analytical phase of urine culture, thus avoiding unnecessary urine culture and waiting time.

KEYWORDS

bacterial morphology, diagnosis, laser flow cytometry, urinary tract infections, urine culture

1 | INTRODUCTION

About two-thirds of women are estimated to go through at least one episode of uncomplicated urinary tract infection (uUTI) throughout their lives.¹ The symptoms of uUTI are usually minor and rarely progress to life-threatening urosepsis. The diagnosis of uUTI is based on clinical

symptoms and a positive urine culture.^{2,3} Although urine cultures provide clues to the types of bacteria and also sensitivities to antibiotics, urine cultures are relatively costly and take 24-48 hours to wait for the results. Further, during collection process, urine specimens can be easily contaminated by periurethral microorganisms, especially in women and the elderly.⁴ The urine cultures with poly-microorganism

growth are regarded as contaminated with rates ranging from 0.8% to 41.6%.⁵ In addition to poly-microorganisms, growth of gram-positive cocci in voided urine of women correlated poorly with catheterized urine culture and were regarded as of little clinical significance due to high probability of contamination.⁶ Inappropriate reporting of contaminated urine cultures can result in poor patient outcome due to sub-optimal therapy.⁷ Therefore, predicting growth of cocci or mixed flora (cocci/mixed) in the pre-analytical phase of urine specimen is essential so as to reduce wasteful manpower, unnecessary medical costs, waiting time, and also to improve patient care.

UF1000i (UF-1000i; Sysmex, Kobe, Japan) stained the bacteria with fluorescence and generated the parameters related to bacteria morphology when the bacteria passed the diode laser beam.⁸⁻¹⁰ By using the scatter diagram of the bacteria forward scatter (B-FSC, Y-axis) and bacteria fluorescent light scatter (B-FLH, X-axis), bacteria in the urine specimen can be classified as either rods or cocci/mixed growth within 30 minutes. Therefore, we did a retrospective review to evaluate the accuracy of the UF1000i in predicting the cocci/mixed growth in midstream voided urine specimens in women visiting urological clinics for uUTI.

2 | METHODS

From October 2014 to October 2015, we retrospectively reviewed adult women (≥ 18 years old) visiting urological clinics at Taipei Tzu Chi General Hospital due to lower urinary tract symptoms suggestive of urinary tract infections, i.e., urinary frequency, urgency, dysuria, and hematuria. Exclusion criteria were those with fever $>38^{\circ}\text{C}$, pregnancy, congenital genitourinary tract anomaly, chronic kidney disease under dialysis, chronic urine retention undergoing urethral catheterization or bladder cancer, and those undergoing immunosuppressant therapy and recent antibiotics use (within 1 week); examinations of urine analysis and urine culture not performed within the same day were also excluded from analysis.

2.1 | Urine analysis with the Sysmex UF1000i

The study site was a hospital-based laboratory which examines >1000 urine specimens every working day. At the clinics, the patients were asked to collect midstream voided volume in a sterile container for urine analysis and urine culture. Then, 10 mL of the urine sample were placed in a urine sediment centrifuge tube 10 mL (SY, Shih-Yung medical instruments Co., Ltd, Taipei, Taiwan) for laser flow cytometry (UF-1000i; Sysmex, Kobe, Japan) within 30 minutes after receiving the specimen. The Sysmex UF-1000i is an automated fluorescence laser flow cytometer using a 635-nm semi-conductor diode laser to classify and count bacteria that does not need to centrifuge the urine specimens. The urine specimens were mixed at 42°C with a special diluent (UFII SEARCH™ - BAC, Sysmex, Kobe, Japan) which increased the permeability of bacteria's cell wall and thus facilitated the staining of the bacterial nucleic acids with fluorescent dye. The bacteria were then classified and counted on the basis of their size and

FIGURE 1 The scatter diagram of the bacteria forward scatter (B-FSC, y-axis, indicating bacteria size) and bacteria fluorescent light scatter (B-FLH, x-axis, indicating nucleic acid contents); bacteria in the urine specimen could be classified as either rods ($<30^{\circ}$, A) or cocci/mixed growth ($>30^{\circ}$, B)

staining characteristics: a specific scatter diagram pattern was generated through the forward scatter (B_FSC) and fluorescent light intensity (B_FLH) emitted from each bacterium, from which bacteria size and the nucleic acid content were, respectively, generated. We only included urine specimens with a bacteria count >357 BACT/ μL for analysis with scatter diagrams. In our previous study, the cut-off point provided an optimal sensitivity (85.9%) and specificity (88.4%) for predicting significant bacteriuria ($\geq 10^5$ cfu/mL). Urine specimens with bacteria counts below >357 BACT/ μL yielded a lower probability of significant bacteria growth count and also made it difficult for interpretation of the diagram due fewer dots on the diagrams. For each analyzed sample, the bacteria scatter diagram was manually classified as either rods (Figure 1A, degree $<30^{\circ}$) or cocci/mixed (Figure 1B, degree $>30^{\circ}$) by agreement between two experienced inspectors (CC Yang and HC Hung). When wide patterns (dots distributed widely both below and above 30° line) were observed on the scatter diagrams, the classification was determined by discussion between the two inspectors about whether the major proportion of dots were below or above the 30° line.

2.2 | Microbiological analysis

Quantitative urine culture was performed using a 1 μL inoculation loop and the commercial chromogenic agar medium (CPS® ID3, Biomerieux, l'Etoile, France). The culture plates were aerobically incubated at 35°C for 18-24 hours and the quantification in CFU/mL, was calculated after multiplying the dilution factor and the colonies on the agar plate. The criteria for positive urine culture was single bacteria with 10^5 cfu/mL or more. In the study, urine specimens with the growth of two or more species of bacteria without a dominant one were considered to be contaminated or mixed growth.

2.3 | The process of selecting urine samples for analysis

Initially, a total of 1680 urine specimens from adult women with symptoms suggestive of uUTI were included for analysis. Samples excluded

from analysis included the following: specimens of urine culture and urine analysis not performed within the same day ($n=264$), recent antibiotics use ($n=82$), urine specimens with severe gross hematuria or cloudy urine that might lead to carry over effect ($n=166$), fungus ($n=8$), *Lactobacillus* spp. ($n=33$), *Gardnerella vaginalis* ($n=9$), and bacteria count less than $357\text{BACT}\times 10^6/\text{L}$ ($n=627$). Finally, 491 urine specimens were included for analysis using the prediction ability of the UF1000i.

2.4 | Statistical analysis

Data were expressed as mean $\pm$ SD and analyzed by commercial statistical software (MedCalc Statistical Software version 16.1, Ostend, Belgium). Nominal or categorical data were compared via a chi-squared test. Agreement was evaluated using kappa statistics and the grade of agreement was classified according to Altman's suggestions (<0.2 : Poor agreement, 0.21 – 0.40 : Fair agreement, 0.41 – 0.60 : Moderate agreement, 0.61 – 0.80 : Good agreement, 0.81 – 1.00 : Very good agreement).¹¹ A P -value of $<.05$ was regarded as statistically significant.

3 | RESULTS

A final total of urine specimens from 491 women (mean age= 58.5 ± 18.5 years) with bacteria count $>357\text{BACT}/\mu\text{L}$ were included for analysis. The analyzed specimens all yielded a bacteria growth of $\geq 10^3\text{cfu/mL}$, which included 376, 10, and 105 specimens with single, 2, and ≥ 3 bacteria species, respectively. Among specimens with single bacteria growth, there were 26 gram-positive cocci (13 *Streptococci* spp., seven *Staphylococci* spp., six *Enterococci* spp.), one gram-positive rods (*Corynebacterium* spp.), and 349 gram-negative rods (273 *Escherichia coli*, 33 *Klebsiella* spp., 29 *Proteus* spp., six *Citrobacter* spp., four *Enterobacter* spp., three *Pseudomonas* spp., and one *Providencia* spp). Specimens with two or more bacteria species were classified as mixed growth in the study.

Agreement levels between rods and cocci/mixed growth between the laser flow cytometry and urine cultures are listed in the Table 1 with a kappa value of 0.58 (95 CI: 0.50–0.66). The sensitivity and specificity of the UF1000i for rods was 94.8% and 57.4%, respectively, and the sensitivity and specificity of the UF1000i for cocci/mixed growth was 57.4% and 94.8%. Through laser flow cytometry, the positive and negative predictive rate for cocci/mixed growth in voided urine was 81.8% and 84.7%, respectively.

The sensitivity of laser flow cytometry for specific bacteria species (classified based on voided urine culture results) are listed in

TABLE 2 The sensitivity of laser flow cytometry for specific bacteria species

Bacterial growth of urine specimens	Number (n)	Sensitivity (%) of laser flow cytometry
Gram (–) rods	349	94.8% (331/349)
<i>Escherichia coli</i>	273	96.3% (263/273)
$\geq 10^5$ cfu/mL	264	96.6% (255/264)
10^3 – 10^5 cfu/mL	9	66.7% (6/9)
<i>Klebsiella</i> spp.	33	93.9% (31/33)
$\geq 10^5$ cfu/mL	30	93.3% (28/30)
10^3 – 10^5 cfu/mL	3	100% (3/3)
<i>Proteus mirabilis</i>	29	93.1% (27/29)
$\geq 10^5$ cfu/mL	26	92.3% (24/26)
10^3 – 10^5 cfu/mL	3	100% (3/3)
<i>Citrobacter</i> spp.	6	100% (6/6)
$\geq 10^5$ cfu/mL	5	100% (5/5)
10^3 – 10^5 cfu/mL	1	100% (1/1)
<i>Enterobacter</i> spp.	4	75% (3/4)
$\geq 10^5$ cfu/mL	4	75% (3/4)
10^3 – 10^5 cfu/mL	0	n/a
<i>Pseudomonas aeruginosa</i>	3	33.3% (1/3)
$\geq 10^5$ cfu/mL	3	33.3% (1/3)
10^3 – 10^5 cfu/mL	0	n/a
<i>Providencia</i> spp.	1	0% (0/1)
Gram (+) rods	1	100% (1/1)
<i>Corynebacterium</i> spp.	1	100% (1/1)
Gram (+) cocci	26	61.5% (16/26)
<i>Streptococci</i> spp.	13	53.8% (7/13)
<i>Staphylococci</i> spp.	7	85.7% (6/7)
<i>Enterococci</i> spp.	6	50% (3/6)
Two bacteria growth	10	50% (5/10)
Mixed growth	105	57.1% (60/105)
$\geq 10^5$ cfu/mL	69	56.5% (39/69)
10^3 – 10^5 cfu/mL	36	58.3% (21/36)

Table 2. For gram-negative bacilli, the sensitivity for each bacteria species was generally high while a lower sensitivity was observed in the *Pseudomonas* species (1/3) and *Providencia* (0/1). The sensitivity for gram-positive bacteria was 59.2%. The sensitivity of the UF1000i for 2 and >2 bacteria species were 50% and 57.1%, respectively. No significant difference was observed in the sensitivity between specimens with 10^5cfu/mL and 10^3 – 10^5cfu/mL ($P>.05$).

TABLE 1 The agreement between the classifications (rods or cocci/mixed growth) of UF1000i and the results of urine culture

Classification by UF1000i	Urine culture	
	Rods (n)	Cocci/mixed growth (n)
Rods (n)	332	60
Cocci/mixed growth (n)	18	81

4 | DISCUSSION

Midstream volume served as a convenient and non-invasive method for obtaining specimens for urine analysis and urine culture.¹² However, the major drawbacks were that urine specimens could be easily contaminated with periurethral, epidermal, perianal, and

vaginal flora and may not, therefore, perfectly represent the true condition of microbiology in the bladder. Although the contamination rate can be reduced with proper attention to techniques for urine collection, transport, preservation, and storage,⁷ there was still a significant proportion (from 0.8% to 41.7%) of urine that was contaminated during the collection process in female patients and resulted in mixed growth results in the urine culture. Gram-positive cocci growth (i.e., *Enterococci* spp. and *Streptococci* spp.) is often observed in the voided urine culture, though these bacteria are considered to rarely cause cystitis in women.⁶ Laser flow cytometry (UF1000i) has been reported to be able to predict cocci/mixed growth or rods in the urine specimen within half an hour by means of morphology parameters. Our study shows that the positive and negative predictive rates for cocci/mixed growth in voided urine were 81.8% and 84.7%, respectively. Within 30 minutes after collection of urine specimen, we were able to learn with high probability whether there was cocci/mixed growth in the specific urine specimen through the laser flow cytometer.

The findings from Gessoni's study⁹ suggest that gram-negative and positive yield significant differences in the parameter of B_FSC and indicate that the UF1000i can differentiate gram-negative bacilli from gram-positive cocci. Possible explanations for the differences between cocci and rods are that cocci usually aggregate or chain, thus resulting in larger and more variable bacteria sizes compared to rods, which in turn leads to a higher number of bacteria forward scatter (B-FSC). Therefore, the distribution of dots of gram-positive bacteria was usually diverse while the patterns of gram-negative bacteria were usually uniform. The scatter diagrams of gram-positive bacteria and mixed growth usually overlapped due to the fact that mixed growth usually contains gram-positive bacteria that creates a wide distribution pattern.^{10,13} Later, it is revised that bacteria could be classified as either rods (<30°, Figure 1A) or cocci/mixed growth (>30°, Figure 1B) based on scatter diagrams.¹⁴ In Geerts et al.'s⁸ study that enrolled patients of both genders, inpatient and outpatient departments, and urological and other specialty clinics with BACT >200/μL, they found that the UF-1000i cannot correctly identify cocci, but the UF 1000i can accurately identify rods with good sensitivity and specificity. However, they observed that most specimens classified by the UF1000i as cocci/mixed growth were rods (68%). In this study, we found that the UF1000i could predict cocci/mixed growth in the pre-analytical phase with poor sensitivity (57.0%), but with good specificity (94.8%), positive predictive rates (81.8%), and negative predictive rates (84.7%). The reasons accounting for the differences between these two studies might be that we chose BACT >357/μL as a cut-off point for the interpretation of the scatter grams. Among specimens with BACT >357/μL, urine culture all grew bacteria with count $\geq 10^3$ cfu/mL and we intabed all these specimens for correlation, whereas Geerts et al.⁸ only included 246 urine positive specimens from 510 specimens (BACT >200/μL) for analysis. Also, mixed growth accounted for 23.4% of the urine culture results in our population, which is higher than the 11.4% in the Geert et al. study.⁸ Through the laser flow cytometry, 57.4% (81/141) of mixed growth were identified and unnecessary cultures were avoided in

female patients suspected of uUTI. However, 18/350 cultures with rods may have been misclassified as cocci/mixed growth. Further, a negative predictive value for cocci/mixed at 84.7% means that 15.3% of the urine specimens may have been recognized as rods and sent for culture, thereby leading to unnecessary wait time and medical costs.

The usefulness of routine urine culture in female patients with uUTI has been questioned because voided urine culture may not perfectly reflect the true bacteriology in the bladder, especially for non-*E coli*-related cystitis.¹² Hooton et al.⁶ reported that the results with enterococci and streptococci poorly correlated with catheterized urine culture, and that only *Staphylococcus saprophyticus* correlated well with the catheterized urine. In our outpatients, 27 out of 491 specimens grew gram-positive bacteria with 13 *Streptococci* spp., seven *Staphylococci* spp., six *Enterococci* spp., and one *Corynebacterium* spp. Gram-positive rods were generally not thought to be the causative organism for uncomplicated urinary tract infections in the women, and no one had *Staphylococcus saproliticus*. However, bacteria resistance to antibiotics has emerged as a more and more severe problem, and bacterial sensitivity to antibiotics offer clinicians information concerning the choice of antibiotics. Therefore, if urine culture is mandatory and the UF-1000i can help identify gram-positive bacteria and mixed growth in the pre-analytical phase of urine culture, this can prevent unnecessary cultures and waiting time.

There are several limitations in our study. The major limitation is that the study is a retrospective review, which is biased in its nature and we did not use a validated symptom score¹⁵ to evaluate the symptoms of the enrolled subjects. Although we excluded urine specimens that were not performed within 4 hours of the collection of the specimen, recent antibiotics use and also the factors that may have compromised the results. A significant patient proportion of specimens yielded mixed growth culture results, so improved patient education may greatly reduce the possibility of mixed growth results. Second, we did not identify the specific bacteria in the mixed growth. The variable combinations of bacteria species in the mixed growth may differ under different clinical conditions and yield different results for morphology identification. The strength of the study is its large sample size of women suspected of having uUTI within a single institution with the same protocol of specimen manipulation. Further studies are still warranted to evaluate the generalizability of the UF1000i in other institutions and populations.

5 | CONCLUSIONS

Through laser flow cytometry, we can predict the growth of cocci/mixed growth in the pre-analytical phase of urine culture, and therefore avoid unnecessary urine cultures and waiting time.

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