Comparing the Performance of the New Fully Automated Urine Particle Analyzer UF-5000 with UF-1000i and Gram Stain in Predicting Bacteria Growth Patterns in Women with Uncomplicated Urinary Tract Infections

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Abstract

Backgroud

To compare the performance of the new flow cytometer UF-5000 with UF-1000i (Sysmex, Kobe, Japan) and Gram stain in predicting the bacterial patterns in urine samples

Methods

Women with symptoms suggestive of urinary tract infection were enrolled. Mid-stream urine sample was collected for gram staining, urine analysis and urine culture. Bacterial patterns were classified though UF1000i (none, cocci bacteria or rods/mixed growth), UF-5000 (none, cocci, rods or mixed growth) and Gram stain.

Results

Among the 102 samples, there were 10 gram-positive cocci, 2 gram-positive bacilli, 66 gram-negative rods, and 24 mixed growth. The sensitivity/specificity of the UF-1000i was 81.8/91.1% for gram-negative rods and 23.5/96.9% for cocci/mixed. The sensitivity/specificity of the UF-5000 was 80.0/88.2% for gram negative rods and 70.0/86.5% for gram-positive cocci.

Conclusions

The UF-5000 demonstrated the good sensitivity and specificity for Gram-negative bacilli bacteria and demonstrated an improved sensitivity for detecting Gram-positive cocci.

Backgrounds

Urinary tract infection is a common infectious disease that leads to a significant health care burden, although most cases are uncomplicated infections. It is estimated that 50-60% of women will go through one or more episodes of uncomplicated urinary tract infection [1]. The uUTI usually leads to minor symptoms and are rarely life-threatening. However, uUTI impaired quality of life (QoL) because of irritative symptoms[2]. The uUTI are diagnosed with positive clinical symptoms and urine cultures. The bacterial species that lead to urinary tract infection included Escherichia coli, klebsiella spp., Enterobacter spp., proteus spp., pseudomonas spp., enterococcus spp., staphylococcus spp., and streptococcus spp. However, the examination of urine cultures takes 1-2 days for results and the high contamination rate (0.8 to 41.6%) during the collection process usually makes interpreting the results difficult or irrelevant[3] 4]. Some experts suggested the results of mixed growth in urine culture may occur among the elderly, immunocompromised, and those with indwelling catheters, HIV, malignancy, and diabetes[5], while these findings are more commonly regarded as contamination[4]. Manual gram staining of urine specimen has shown a 90% sensitivity when utilized to diagnose bacteriuria, however it is laborious and time consuming[6]. Therefore, a rapid, automatic, reliable and cheaper screening test to differentiate gram positive, negative or mixed growth in the urine specimen is required to reduce labor, unnecessary medical
costs, waiting time and also to help clinicians improve patient care. Previous studies have examined the efficacy of the UF1000i (Sysmex UF-1000i; Sysmex Corporation, Kobe, Japan) in differentiating bacilli from cocci/mixed growth[7]. However, the UF1000i cannot differentiate cocci from mixed growth. Recently, a new model of urine particle analyzer, the UF5000 (Sysmex Corporation, Kobe, Japan), was introduced where cocci are labeled separately from mixed growth bacteria[8, 9]. Therefore, we did a prospective study to compare the accuracy of the gram staining, the UF1000i and the UF5000 in differentiating bacterial growth patterns (gram positive, negative or mixed growth) in midstream voided urine specimens in women visiting urological clinics for uUTI.

**Methods**

The study was approved by the Institutional Review Board in our hospital. From July, 2016 to June, 2019, we prospectively enrolled adult women (20 to 80 years old) visiting our clinic presenting with symptoms suggestive of urinary tract infection without fever >38°C, urolithiasis, pregnancy, congenital urinary tract anomaly, end stage renal disease under dialysis, neurogenic bladder under urethral catheterization, bladder cancer history, patients who were immunocompromised and recent antibiotics use (within seven days). After written informed consents were signed, patients were asked to complete a questionnaire including baseline characteristics (age, medical history including diabetes or hypertension, childbirth, previous abdominal surgery). They were also asked to complete a urinary tract infection symptoms assessment (UTISA[10]) which included 7 symptom categories and 7 quality of life categories, with scores for each assessment ranging from 0 to 3. Patients with total symptom scores of 4 or more were regarded as positive symptoms of urinary tract infection. At the clinics, the patients were asked to collect midstream voided urine in a sterile container for manual gram staining, routine urinalysis and urine culture. A study nurse instructed the patients on proper collection technique to attempt to reduce the contamination rate. Only specimen with a bacterial growth of \( \geq 10^3 \) cfu/mL were included for comparison of bacterial growth pattern differentiation. From the sterile collection cup, 10 ml of sample was poured into urine sediment centrifuge tube (SY, Shih-Yung medical instruments Co., Ltd, Taipei, Taiwan) for automated urine particle analysis (Sysmex UF-1000i; UF-5000, Sysmex Corporation, Kobe, Japan) within half an hour after receiving the specimen. The gram staining of urine specimens was classified as gram positive, negative or mixed by two experienced clinical laboratory scientists with more than 10 years in the field. For each analyzed sample, the bacteria scatter diagram was classified as rods, cocci/mixed growth or none on UF1000i. The bacteria scatter gram was classified as gram positive, negative, mixed growth or none on UF5000[9, 11].

**Gram staining**

The centrifuged urine from the urine sediment preparation was used to make slides for Gram staining. These slides were air dried, fixed with heat, and then stained using the Gram stain procedure. Slides were assessed for the presence of bacteria and the staining characteristics were further described. Slides with bacteria were subsequently evaluated for bacterial morphology and whether these bacteria were Gram
positive or Gram negative. Slides were classified as positive for bacteriuria if \( \geq 1 \) bacteria/HPF was noted. Then, the specimens were classified as gram positive, negative, mixed growth or none.[6]

**Microbiological analysis**

A 1 µl inoculation loop was applied to the commercial chromogenic agar medium (CPS® ID3, Biomerieux, l’Etoile, France) for urine culture. The culture plates were incubated at 35°C for 18-24h aerobically. The quantification process of bacteria were by multiplying the dilution factor and the colonies on the agar plate. The growth of more than 2 species of bacteria within the urine culture without a dominant one were regarded as contaminated or mixed growth.

**Statistical analysis**

The data within the manuscript was expressed as mean ± standard deviation. We used MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium;https://www.medcalc.org; 2019) for statistical analysis. Nominal or categorical data were compared with a \( X^2 \) test. Ordinal data were compared with Mann-Whitney test. Continuous data were compared with independent t-test, respectively. Agreement between two methods were evaluated with kappa statistics. The grading of agreement complied with Altman’s recommendations[12] (<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). A p-value of less than 0.05 was considered as statistically significant.

**Results**

There were 85 patients with no UF5000 of UF1000i interpretation and 1 specimen with no growth on culture were excluded for final comparisons. A total of 102 urine specimens from 102 women (mean age = 58.5 ± 18.5 years) with UTISA \( \geq 4 \) and bacteria growth \( \geq 10^3 \text{cfu/mL} \) were included for analysis. Table 1 summarizes the baseline characteristics of the 102 patients. The analyzed specimens included 10 gram-positive cocci, 2 gram-positive bacilli, 66 gram-negative rods, and 24 specimens with two bacteria species or more that were regarded as mixed growth (Table 2). Gram-positive bacilli (Lactobacillus species) were excluded for analysis of agreement. Among specimens with single bacteria growth, there were Gram positive cocci (2 *Streptococci spp.*, 3 *Staphylococci spp.*, 1 *Enterococci spp.*, 2 Group B Streptococci, 2 unclassified G(+)cocci) and Gram negative rods (53 *Escherichia coli*, 5 *Proteus spp.*, 4 *Klebsiella spp.*, and 4 *Citrobacter spp.*).
# Table 1
baseline characteristics of the included patients

|                      | All  | Menopause (+) | Menopause(-) | P value |
|----------------------|------|---------------|--------------|---------|
| n = 102              |      | n = 56        | n = 44       |         |
| Age (years, ±SD)     | 49.56 ± 16.57 | 62.32 ± 7.55  | 33.81 ± 9.78 | P < 0.001 |
| Diabetes Mellitus, n (%) | 12 (11.7)  | 10 (17.8)     | 2 (4.5)      | P = 0.042 |
| Hypertension, n (%)   | 21 (20.5) | 20 (35.7)     | 1 (2.2)      | P < 0.001 |
| Childbirth, n (%)     | 65 (63.7) | 49 (87.5)     | 16 (36.6)    | P < 0.001 |
| Hysterectomy n(%)     | 18 (17.6) | 17 (30.3)     | 1 (2.2)      | P = 0.001 |
| Abdominal surgery history, n (%) | 10 (9.8) | 8 (14.2) | 2 (4.5) | P = 0.109 |
| Day 0 UTISA score (mean ± SD) | 10.71 ± 3.81 | 10.41 ± 3.73 | 11.09 ± 4.01 | P = 0.383 |
Table 2
The sensitivity of gram stain, UF1000i and UF5000 for specific bacterial species

| Bacterial growth of urine specimens | Number (n) | UF1000i | UF5000 | Gram stain |
|------------------------------------|------------|---------|--------|------------|
| **Gram (-)**                       |            |         |        |            |
| Escherichia coli                   |            |         |        |            |
| ≧ 10^5 cfu/mL                      | 39         | Rods = 38/cocci/mixed = 1 | G(-) = 31/G(+) = 3/none = 1/mixed = 4 | G(-) = 34/None = 5 |
| 10^3-10^5 cfu/mL                   | 10         | Rods = 3/cocci/mixed = 1/none = 6 | G(-) = 6/G(+) = 3/none = 1 | G(-) = 4/mixed = 2/None = 4 |
| Klebsiella spp.                    |            |         |        |            |
| ≧ 10^5 cfu/mL                      | 3          | Rods = 3 | G(-) = 3 | G(-) = 2/G(+) = 1 |
| 10^3-10^5 cfu/mL                   | 1          | None = 1 | None = 1 | None = 1 |
| Proteus mirabilis                  |            |         |        |            |
| ≧ 10^5 cfu/mL                      | 2          | Rods = 2 | G(-) = 2 | G(-) = 1/None = 1 |
| 10^3-10^5 cfu/mL                   | 2          | Rods = 1/none = 1 | G(-) = 2 | G(-) = 1/None = 1 |
| Citrobacter spp.                   |            |         |        |            |
| ≧ 10^5 cfu/mL                      | 3          | Rods = 2/none = 1 | G(-) = 3 | G(-) = 2/None = 1 |
| 10^3-10^5 cfu/mL                   | 1          | None = 1 | G(-) = 1 | None = 1 |
| **Gram (+)**                       |            |         |        |            |
| Streptococci spp.                  | 1          | cocci/mixed = 1 | G(+) = 2 | G(+) = 1 |
| Staphylococci spp.                 | 3          | Rods = 1/cocci/mixed = 1/none = 1 | G(+) = 3 | G(+) = 2/None = 1 |
| Enterococci spp.                   | 1          | Rods = 1 | G(+) = 1 | G(+) = 1 |
| Group B Streptococci               | 2          | None = 2 | G(+) = 1/none = 1 | G(+) = 1/None = 1 |
| G(+)cocci                          | 2          | None = 2 | None = 2 | None = 2 |
| Lactobacillus species              | 2          | cocci/mixed = 1/none = 1 | G(+) = 1/none = 1 | None = 2 |
| **Mixed growth**                   |            |         |        |            |
| Bacterial growth of urine specimens | Number (n) | UF1000i | UF5000 | Gram stain |
|------------------------------------|-----------|---------|--------|------------|
| ≥ 10⁵ cfu/mL                      | 3         | Rods = 1/cocci/mixed = 1 | G(+) = 1/G(-) = 1/mixed = 1 | Mixed = 2/None = 1 |
| 10³-10⁵ cfu/mL                    | 19        | cocci/mixed = 3/None = 16 | G(+) = 5/G(-) = 3/None = 11 | G(-) = 1/G(+) = 1/mixed = 2/None = 15 |

Gram stain

There were only 97 specimens with gram stain results available. Of them, 29 urine specimens were classified as negative. Agreement levels between the results of gram stain and urine cultures are listed in the Table 3 with a kappa value of 0.48 (moderate, 95%CI: 0.36 to 0.60). The sensitivity and specificity of the gram stain for gram negative bacteria was 80.6% and 96.7%, respectively, and the sensitivity and specificity of the gram stain for cocci was 60% and 100%. The sensitivity and specificity of the gram stain for mixed growth was 18.2% and 97.4%, respectively.

Table 3
Agreement levels between the results of gram stain and urine cultures

| Gram stain |
|------------|
| **Urine culture** | GNB | GPC | Mixed | none |
| GNB         | 50  | 0   | 2     | 10   |
| GPC         | 0   | 6   | 0     | 4    |
| Mixed       | 1   | 0   | 4     | 16   |

GNB: gram negative bacilli

GPC: gram positive cocci

Mixed: mixed growth

UF1000i

Agreement levels between the results of the Gram-negative bacilli UF1000i and urine cultures are listed in the Table 4 with a kappa value of 0.49 (moderate, 95%CI: 0.38 to 0.61). The sensitivity and specificity of the UF1000i for rods was 81.8% and 91.1%, respectively, and the sensitivity and specificity of the UF1000i for cocci/mixed growth was 23.5% and 96.9%, respectively.
Table 4
Agreement levels between the results of UF1000i and urine cultures

| Urine culture | Rods | Cocci/Mixed | None |
|---------------|------|-------------|------|
| Rods          | 54   | 2           | 10   |
| Cocci/Mixed   | 3    | 8           | 23   |

UF5000

Agreement levels between the results of the UF5000 laser flow cytometry and urine cultures are listed in the Table 5 with a kappa value of 0.46 (moderate, 95CI: 0.34 to 0.58). The sensitivity and specificity of the UF5000 for GNB was 80.0% and 88.2%, respectively, and the sensitivity and specificity of the gram stain for cocci was 70% and 86.5%.. The sensitivity and specificity of the UF5000 for mixed growth was 4.5% and 94.9%, respectively. For specific gram positive cocci, the UF 1000 identified all *staphylococci spp.* (n = 3), *enterococci spp.* (n = 1), *streptococci spp.* (n = 3), except one Group B Streptococci and two gram (+) cocci (10³ and 2 × 10³ cfu/mL, respectively).

Table 5
Agreement levels between the results of UF5000 and urine cultures

| Urine culture | GNB | GPC | Mixed | None |
|---------------|-----|-----|-------|------|
| GNB           | 52  | 6   | 5     | 2    |
| GPC           | 0   | 7   | 0     | 3    |
| Mixed         | 4   | 6   | 1     | 13   |

For specific gram negative bacilli, the UF5000 identified all *Proteus spp.* and *Citrobacter spp.* except one *Klebsiella spp* (3 × 10³ cfu/mL). However, the UF5000 identified only 37 of 49 *E. coli*.

Discussion

This is the first prospective study that compares the efficacy of automated urine flow cytometry system (UF1000i, UF5000), gram stain and urine cultures in urine specimens of women with uncomplicated
The results showed that the UF5000 kept a good sensitivity (80.0%) in identifying gram negative bacteria with an acceptable specificity (88.2%). With regard to gram positive bacteria, the UF5000 outperformed the UF1000i in detecting gram positive cocci (UF5000 sensitivity: 70% and specificity: 86.5%) with good specificity which was comparable to gram staining (sensitivity: 60% and specificity: 100%). However, the sensitivity of the UF5000 for identifying mixed growth bacteria was poor.

The UF-5000 is an automated urine analyzer produced by Sysmex Corporation, which performs flow cytometry analysis with a higher level of accuracy and more precise data [13]. Several previous studies investigated the legacy models of automated urine particle analyzers (including the UF-500i and the UF-1000i) for screening urine cultures, while few studies have evaluated the ability of the newer UF5000 in differentiation of bacteria growth patterns. Compared with the legacy systems, the current study showed that the UF5000 kept the comparable sensitivity and specificity for GNB (80.0% and 88.2%, respectively). For specific bacteria (Table 2), the current results revealed that the UF5000 will identify *Klebsiella spp.* *Proteus spp.*, and *Citrobacter spp.* while only identifying 37 out of 49 *E coli*. However, the retrospective study by Kim et al [11] showed good performance of the UF5000 in identifying *E coli*. Further studies are required to check the performance of the UF5000 in identifying *E coli*. As for Gram-positive bacteria, the UF 5000 showed high sensitivity and specificity for *Enterococcus spp.* However, the sensitivity for Streptococci spp. was much lower. In our study, UF 5000 identified all staphylococci spp. (*n* = 3), enterococci spp. (*n* = 1), streptococci spp. (*n* = 3), except one Group B Streptococci (2*104 cfu/mL) and two gram (+) cocci (103 and 2 × 103 cfu/mL, respectively). With regard to gram positive bacteria, the UF5000 outperformed the UF1000i in detecting gram positive cocci which was comparable to gram staining.

Gram staining is associated with a sensitivity rate of 88%, a specificity rate of 95%, a negative predictive value of 96%, and a positive predictive value of 84% for identifying bacteriuria. [6, 14] For differentiating bacterial growth patterns, gram stain yielded good sensitivity and specificity for gram negative (80.6% and 96.7%, respectively) and gram positive bacteria (60% and 100%, respectively). Although a real-time reporting of gram stain could reduce the blind initiation of antibiotics, and thus prevent unnecessary expenditure and drug treatment, gram staining is time consuming and labor-intensive. The UF-5000 offers comparable efficacy and a faster and far easier way to provide the same information as compared to the classic method of Gram staining.

Detecting Gram positive bacteria has significant clinical implications. The most commonly isolated Gram-positive uropathogens are *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*. One review published in the literature suggests that urologic diseases involving Gram-positive bacteria may be easily overlooked due to limited culture-based assays typically utilized for urine in hospital microbiology laboratories [5]. However, Hooton et al [12] found that only *Staphylococcus saprophyticus* correlated well with the catheterized urine. However, enterococci and streptococci correlated with catheterized urine culture poorly. Therefore, patients with gram positive bacteria shown on the UF5000 may not have uUTI by classical GNB and may not need empirical antibiotics. Second, in patients with Gram positive cocci determined by UF5000, urine culture is recommended and antibiotics
targeting Gram positive bacteria, instead of empirical ones for gram negative bacteria. In this way, we may avoid unnecessary waiting times, overuse of antibiotics and increased medical costs. Third, immediately identifying Gram positive bacteria in ascites[13], cerebral spinal fluids[14] and pleural fluids may help clinicians make appropriate and timely antibiotic choice in these life threatening infections. More clinical studies to explore the use of the UF-5000 in the situations is encouraged.

About 21.6% of urine cultures revealed mixed growth (n = 22, 21.6%) and lactobacillus (n = 2, 1.9%) which were regarded as contamination due to improper collection, transportation, preservation, and storage. As the study included female participants only, and this may explain the relatively higher contamination rate. Because the short urethral length and the urethra meatus is proximal to the vagina and anus, urine specimen from women were more easily contaminated than men. Our study shows that the sensitivity and specificity of the UF5000 for mixed growth was 4.5% and 94.9%, respectively. Further improvement of laser flowcytometry in identification of mixed growth could help health care and avoid the waste of time, labor and money.

There existed some limitations in our study. Although the study is a prospective study that compared the efficacy of the three methods (gram stain, the UF1000i and the UF 5000i), the major limitation is that the number of samples was limited. Second, a significant proportion of patient specimens yielded gram negative bacteria and mixed growth culture, so the evidence supporting the promising efficacy of the UF5000 in detecting gram positive bacteria is limited due to the low number of specimens with gram positive bacteria. The strength of the study is its prospective nature of the study that compares two automated urine particle analyzers. Further studies are still warranted to evaluate the generalizability of the UF5000 in a larger subset of patients, institutions and populations. Also, the role of the UF5000 in detecting bacterial patterns in the other type of specimens, i.e. ascites, cerebral spinal fluids and pleural effusion, should be investigated further.

**Conclusions**

The UF-5000 demonstrated potential utility for the rapid screening of bacterial morphology, which correlated well with the legacy analyzer UF1000i for GNB bacteria while showing improved sensitivity for detecting Gram-positive cocci.

**Abbreviations**

GNB
Gram negative bacilli

UTISA
urinary tract infection symptoms assessment score

uUTI
uncomplicated urinary tract infection
Declarations

Ethics approval and consent to participate:

The study was approved by the Institutional Review Board in Taipei Tzu Chi Hospital: IRB No:05-FS02-024

Written informed consents were obtained

Consent for publication: approved by the IRB

Availability of data and materials: as requested to the corresponding author

Competing interests: none

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Authors' contributions

SSY, SJC: Project development, Data Collection, Manuscript writing

YSC, CCY: Data collection

SSY, SJC: Manuscript writing

all authors have read and approved the manuscript

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