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Original Article

Comparison of Urine Culture and Flow Cytometric Methods for Detecting Bacteriuria by Using a Simulation Model

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ABSTRACT

Objective: We aimed to simulate and assess a new screening model to determine and exclude culture negative urine samples before culturing for patients with preliminary diagnosis of urinary tract infections (UTIs). This prospective and single-center research included a simulation model that studied in a central laboratory between March and April 2020. All samples studied fluorescent flow cytometry (FC) analyzer and then inoculated to medium.

Methods: Simulations of infected urine were created by mixing certain amounts microorganisms with the urine. Standard *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* strains, one *Lactobacillus* spp., and one *Staphylococcus epidermidis* clinical isolate were used in the study. After the dilution process, 42 infected urine samples were analyzed using UF5000i FC device and urine culture method. Correlation between the methods (culture and FC) for bacterial counts was assessed with the in-class correlation coefficient and Spearman correlation coefficient.

Results: A significant agreement was observed between the methods only for the urine dilution containing 10^5 CFU mL^{-1} pathogen.

Conclusion: The flow cytometric system failed to predict bacteriuria and the risk of urinary tract infection in our simulation model. Further research in combination with other parameters is needed to see the real power of flow cytometric methods for screening UTIs.

Keywords: Urinary tract infections, urine culture, flow cytometric method, simulation model

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections in patients attending hospitals and healthcare settings. These types of infections generally respond rapid to antibiotic treatment; as a result, it is important to rapidly diagnose and treat these infections.[1] The gold standard for etiologic diagnosis of UTIs is urine cultures; however, it generally takes 48 hours to perform urine culture and then microbiological identification of bacteria.[1–4] The isolated pathogen then has antibiogram performed after culture processes to ensure the clinician begins the patient on an appropriate antibiotic.[1] Urine samples are one of the most commonly used samples in clinical laboratories, and more than half of cultures provide negative results.[5] As a result, screening methods identifying and excluding clinically insignificant bacteriuria gain importance for urine samples.[6] There are many screening tests used to research the presence of bacteria and/or leukocytes in urine for UTI diagnosis. The most commonly used tests are gram staining of urine, nitrite test for enteric bacilli, leukocyte count in urine, and the identification of pyuria with leukocyte esterase activity.[1,2] In recent years, cytometric methods have come to the fore for UTI screening with the development of flow cell count devices. Flow cytometry (FC) method ensures differentiation of bacteria, leukocytes, erythrocytes, and other particles in urine. The Sysmex UF-5000i (Sysmex Corporation, Japan) is an automated latest generation FC device that has a second channel that may identify bacteria, and this device is proposed to have high sensitivity and specificity.[7] The FC UF-5000 analyzer used in our study was produced as a third generation fully automatic urine device. This analyzer may differentiate 17 diagnostic cell parameters and perform cell counts; additionally, the integrated body fluids (BFs) mode may classify and count seven diagnostic parameters. The system uses fluorescent FC technology at 480 nm wavelength and can perform two different analyses with a semiconductor laser and hydrodynamic focusing of surface (SFch) and core (CRch) analyses. Particles are stained with specific fluorochromes for both nucleic acid and surface in the device and sent into the laser. Counts and classifications are determined according to the emerging signals. These signals are, in order, signals from forward scattered light (FSC), side scattered light (SSC), side fluorescent light (SFL), and the new depolarized side scattered light (DSS). Specific algorithms analyze these light signals, and particles are differentiated into categories as identified. Due to different stain intake into the cell wall structure, FSC, SFL, and SSH light signals are used to differentiate gram-negative and

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gram-positive bacteria. Based on this, the UF-5000 system is reported to be able to image gram morphology of bacteria, and this situation is described as Bact Info flag in the device.[8] When the UF series is compared with older versions, the sensitivity and specificity features for bacterial identification have been enhanced with technological innovations. Additionally, the UF-5000 is better for fungal detection.[9] Though devices performing cytometric counts are reliable and can provide rapid results, fragmented leukocytes and dead bacteria affect the sensitivity of the test. Additionally, as noninfectious inflammation causes may create pyuria, leading to similar symptoms, urine culture is definitely necessary for these types of patients.[1] Etiologic bacterial UTI diagnosis is made with urine culture.[10] After incubation of noninvasive urine culture samples (midflow urine or Foley catheter), observation of $\geq 10^4$ CFU mL^{-1} colony counts on media or $\geq 10^3$ CFU uropathogen mL^{-1} for women from 14 to 30 years is accepted as significant for UTI.[2] The gold standard for UTIs is to take a urine culture before beginning antibiotic treatment.[2,3]

In this study, we aimed to simulate and assess a new screening model to determine and exclude culture negative urine samples before culturing for patients with preliminary diagnosis of UTI by clinicians. Preconditions for our screening model were high sensitivity of the screening test to prevent false negative classification of patient samples with notable bacteriuria and high specificity to prevent unnecessary culture request. In our study, we researched the detection adequacy of the Sysmex UF-5000 FC analyzer as a screening test for bacteriuria (or UTI). This test was compared with the gold standard of urine culture to assess the potential to use the FC system as a screening test before implementing the labor-intensive and costly test of urine culture.

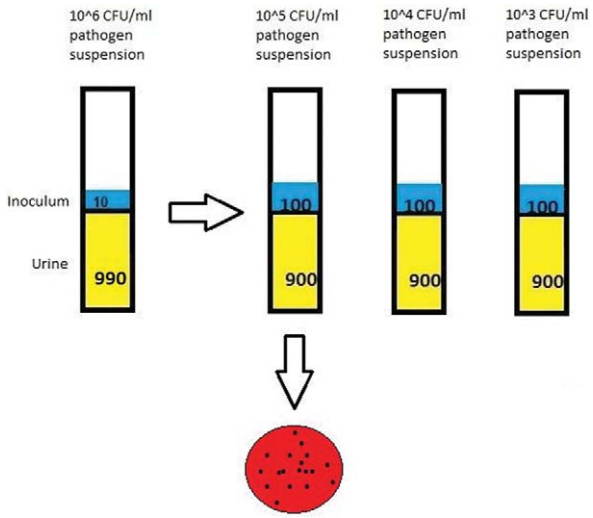
METHODS

This study used standard bacterial and fungal strains with samples from a healthy volunteer without UTI. Urine obtained from a healthy volunteer was used for the research within 1 hour. Simulations of infected urine were created by mixing certain amounts of the following standard bacterial strains with the urine to be tested. In this study, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 standard strains, one *Lactobacillus* spp., and one *Staphylococcus epidermidis* clinical isolate were used. After the dilution process, 42 urine samples (seven strains × six dilutions) were tested and assessed with two separate methods. Ethical committee approval was received from the Clinical Research Ethics Committee of Gaziantep University (December 18, 2018; 2018/381).

Main Points

- The use of flow cytometry in hospitals can bring lots of advantages, but it is a method that requires times, is laborious, and is expensive.
- We created a simulation model of UTI by creating artificial bacteriuria in our study and found an agreement between the flow cytometric method and urine culture only for the tubes containing 10^5 CFU mL^{-1} pathogen.
- In our simulation model, the flow cytometric system failed to predict bacteriuria; however, further researches such as the fluorescence in situ hybridization technique are needed in this space.

Figure 1. Preparation of pathogen-containing urine dilutions for the urine infection simulation.



Inoculation and Dilution

First, 1 mL of urine was placed in a sterile glass tube and 10 µL was discarded. At this point, a 0.5 McFarland = 10^8 CFU mL^{-1} bacteria (*E. coli*) suspension was prepared in sterile physiological serum (0.9% NaCl). Then, 10 µL of the bacterial solution was mixed with 990 µL urine to lower bacterial density to 10^6 CFU mL^{-1} (1/100). The urine-bacterial solution obtained after this stage was included in 10-time serial dilution studies and after dilutions, urine samples with 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 CFU mL^{-1} bacterial density were obtained (Figure 1).

Urine Culture

The first solution obtained from the pathogen-urine suspensions in the microbiology laboratory was discarded, and a 10 µL urine with calibrated essence from the second, third, fourth, fifth, and sixth was appropriately inoculated in 5% sheep blood agar and eosin methylene blue agar culture plates. The colonies growing on the culture plates were manually counted after 24 hours of incubation at 35°C and investigated in terms of contamination. Additionally, gram staining was performed on the growing colonies.

Flowcytometric Cell Count

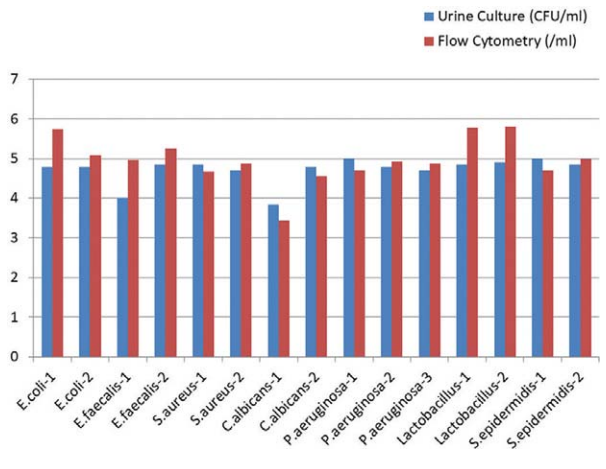
A Sysmex UF-5000i (Sysmex Corporation, Japan) system was used to count pathogen microorganisms in urine. The capacity of the Sysmex UF-5000 system is 105 uncentrifuged urine samples. A total of 2 mL minimum volume is studied, with 0.6 mL urine used in BF mode and 0.45 mL fluid required for aspiration volume in automatic state mode. In our study, 500 µL from each urine tube was investigated in the FC system at the same time. After culture and FC tests were performed with the standard *E. coli* strain first, other standard bacterial and fungal strains were examined.

Table 1. Intraclass Correlation Coefcient (ICC) and Spearman Correlation Coefcient (r) Results

	Spearman r	ICC (95% CI)	*P*
105	r = 0.220; *P* = .430	0.46 (0.05-0.78)	.036*
104	r = 0.251; *P* = .367	0.42 (-0.10 to 0.76)	.054
103	r = 0.077; *P* = .784	0.06 (-0.45 to 0.54)	.413
102	r = -0.257; *P* = .355	-0.22 (-0.65 to 0.31)	.792
101	r = -0.422; *P* = .117	-0.17 (-0.62 to 0.36)	.739

ICC, intraclass correlation coefficient; CI, confidence interval.
*$P < .05$ was considered statistically significant

Figure 2. The number of microorganisms detected by two methods in the simulation of urine containing 10^5 CFU mL^{-1} pathogen (log 10).



Statistical Method

Correlation between the two methods (culture and FC) for bacterial counts was assessed with the in-class correlation coefficient (ICC) and Spearman correlation coefficient (r). All statistical analyses used Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). *P* values <.05 were accepted as statistically significant.

RESULTS

In our study, a significant agreement was observed between the two methods only for the urine dilution containing 10^5 CFU mL^{-1} pathogen (Table 1 and Figure 2).

However, the ICC value was found to be as low as 0.46 (moderate agreement). Regarding other dilutions, no significant correlation was observed between the results of both two methods (Table 1).

DISCUSSION

Though urine culture is the gold standard for the detection of UTIs, the labor-intensive nature and need to wait for at least 24 hours to obtain results have motivated researchers to search for more rapid diagnostic tests.[11,12] For UTIs, a screening test to differentiate negative samples, especially to prevent negative samples not containing pathogens from being included in unnecessary culture processes, will be beneficial from an economic aspect. In Turkey, a 2018 study by Üzmez et al.[13] compared the FC method with the culture method, and they reported 31% of samples coming from all clinics did not need urine culture processes according to their results. They stated that 29.3% of samples from the urology clinic did not require urine culture processes. In this study, they identified that 54 out of 73 patients without proliferation in culture (74%) were positive in the FC system.

De Rosa et al.[8] compared the diagnostic performance of the UF-5000 system with urine culture in 2018. They found that the negative predictive value (NPV) and positive predictive value (PPV) of the UF-5000 system were 87.6 and 66.6 at a cutoff of 40 WBC count μL^{-1} for all samples, respectively. They concluded that the UF-5000 represented a rapid and reliable method for ruling-out UTI and stated that it offers the chance to detect gram negative bacteria in very high agreement with urine culture. However, in their study, they analyzed the results in combination with flowcytometric WBC count results (not alone). That is because they reported UF-5000 system as a reliable method. In another study to rapidly discriminate culture-negative urine specimens from patients with suspected UTI with the UF-5000, researchers obtained a sensitivity of 97.8%, a specificity of 74.6%, a PPV of 46.9%, an NPV of 99.3%, and an agreement of 78.9% with the culture method and stated that it reduced unnecessary urine culture by 61%.[14] However, both cutoff values for bacteria and WBC (bacteria less than 30 μL^{-1} and/or WBC less than 200 μL^{-1}) were included in this study to increase the power of the screening test. In another study, researchers investigated "a new technology to support microbiologists" for interpretation of suspected UTIs using the UF-5000.[15] They used the parameters of squamous epithelial cells, WBC, and conductivity of urine for prediction of UTIs and reported a sensitivity equal to 100% and a specificity equal to 94%, with a total of 69 false positives. They recommended further studies to use this method for rapid detection of UTIs. There are promising studies for rapid detection of UTIs; however, the successful ones are focused on evaluating multiple urine parameters all together. In a recent study, Kim et al.[16] compared the UF-5000 system with urine culture for diagnosis of UTIs. They reported that using a cutoff value of <15 bacteria μL^{-1} to determine whether or not to culture samples, 50.9% of samples were below the cutoff, 94.8 and 99.5% of which presented $<10^4$ and $<10^5$ CFU mL^{-1} of bacterial growth, respectively. They presented this case as a positive result in their study. However, since we can diagnose UTIs at the growth level of 10^3 and 10^4 CFU mL^{-1} in clinical microbiology laboratories, we disagree with the idea that UF-5000 system can be used to eliminate negative samples.

In our study, we investigated only one parameter that is the bacterial count in urine indicating bacteriuria. Although only bacteriuria is not enough to diagnose UTI, it gives a clue of UTI

in combination with physical examination and hematological parameters. So, we created a simulation model of UTI by creating artificial bacteriuria in our study. We found an agreement between the two methods for the tubes containing 10^5 CFU mL^{-1} pathogen. But when the pathogens in the urine decreased, we could not find agreement between the two methods. Significant agreement was observed between the two methods only for the urine dilution containing 10^5 CFU mL^{-1} pathogen (Table 1 and Figure 2) in our study. However, the ICC value was found to be only 0.46 (moderate agreement). No significant correlation was observed between the results of both methods. When we analyze our results, the UF-5000 system gave high positives to almost every urine sample. That is why false negativity is low in the analysis, but false positivity is too high. For example, the UF-5000 system gave a result of 4.96 ± 0.59 ($\log^{10}$) with a bacterial density of 10^5 (5 $\log^{10}$) CFU mL^{-1}. So, we cannot evaluate FC as an appropriate test for diagnosis of UTIs. As a limitation of our study, we did not use clinically obtained UTI samples from our hospital. We prepared the infected urine samples artificially since we wanted to adjust the number of bacteria to definite concentrations for different urine simulation models.

CONCLUSION

The flow cytometric UF-5000 system failed to predict bacteriuria and the risk of UTI in our infection simulation model. However, using the UF-5000 system in combination with other parameters such as WBC and conductivity, we may obtain more promising results in the future. Further research is needed to see the real power of FC methods for the diagnosis of UTIs.

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