Original Article

DOI: 10.6966/EDMJ.202403_11(1).0002

Evaluation of Urine Sediment in Automated Urinalysis System to Screen for Further Cultures

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Objective: In the clinical microbiology laboratory, up to 80% of urine cultures for urinary tract infections (UTIs) are often negative. We investigated whether the use of the flow cytometry cut-off value may reduce the number of urine samples that need to be cultured when used as a quick screening method; to rule out bacteriuria using Sysmex UF-1000i.

Methods: To screen for bacteria or leukocytes in 215 urine samples, flow cytometry and urine culture were used. Using a receiver operator characteristic (ROC) curve analysis, we investigated the ideal cut-off value for additional bacterial culture. Researchers were able to show the significance of selecting the best cut-off values for urine cultures, using urine samples of 7,550 patients with suspected UTIs from the emergency room of the E-Da Hospital.

Results: In 215 urine samples, ROC curve analysis identified bacteria count 100 bacterial (BACT) μL or leukocyte count 10 white blood cell (WBC) μL as potential indicators of bacterial growth. When the bacterial count cut-off was 100 BACT μL, a urine sample had a negative predictive value of 91.2% and a sensitivity of 95.2%, which meant that in around 37.9% of instances, a urine sample could be reported as negative without a culture. Due to an increase in growth rate from 43.3% to 86% and a decrease in the significant culture negative rate from 43.3% to 8.9%, the adoption of cut-off predictors can prevent 2,862 samples, out of 7,550 urine samples, from being further plated.

Conclusions: When urine samples are analyzed for UTIs, several culture-positive results are discovered. The effort and response time involved with culture plating are reduced by using flow cytometry to rule out urine samples quickly and effectively, without detectable bacterial growth.

Key words: urinary tract infections, automated urinalysis system, cut-off of urine sediment count, urine culture

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Received: August 19, 2022 Accepted: January 9, 2023

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Introduction

A urinary tract infection (UTI) is an infection of the bladder and related structures by microbes. The cause of UTI may include bacteria or fungi.[1] Urinary tract infections (UTIs) result in more than 8.1 million visits to a doctor each year and 80 percent of UTI patients will experience recurrences.[2] A UTI is brought on by pathogenic germs that rise from the perineum. Women are far more prone to UTIs than men since they have shorter urethras. UTIs may be classified according to the clinical course, the underlying disease, and site of the infection.[3] Complicated UTI is defined as an infection of an underlying disorder that may interfere with urination. UTI testing may be carried out if it is detected based on a patient's symptoms and physical examination. Lab testing can both identify germs that cause infections and diagnose UTIs, which aids doctors in determining the most effective course of treatment. Urinalysis and urine culture with antibiotic susceptibility testing are the two most often used techniques to identify UTIs. The most accurate way to determine whether there are bacteria in urine is to perform a urine culture, which is a laborious and time-consuming method with a turnaround time of up to 48 hours, and generally a great amount of samples are culture-negative.[4-6] Over the past years, urine flow cytometry (UFC) has been implemented by several laboratories as part of their routine urine diagnostics to increase the sensitivity and specificity of urine test strips for urinary tract infection screening.[4-9]

Escherichia coli (E.coli) causes up to 90 percent of UTIs, but other types of bacteria, such as *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Candida spp,*[10] may cause UTIs too.[11] The sensitivity and specificity of urine test strips for detecting urinary tract infections can be improved by routine urinalysis assessment of leukocytes and bacteria in urine sediment analysis. Small amounts of bacteria are often present in normal asymptomatic human urine ($< 10^3$ colony-forming unit [CFU]/mL). For midstream urine and catheterization samples without antibiotics, when the colony count is greater than 10^5 CFU/mL, it has clinical significance. In samples with antibiotics, single catheterization, and suprapubic aspiration, even low bacterial concentrations ($\geq 10^3$ CFU/mL) were clinically significant. The diagnosis of urinary tract infections heavily relies on colony counts. As a result, laboratories must use quantitative inoculation techniques when handling samples, and the results of quantitative counts must also be included when reporting results.[12]

Materials and Methods

Study samples

A total of 7,765 sterile urine samples with UTI symptoms were collected, between January and July 2017, from the Emergency Department of E-Da Hospital and sent for particle detection using flow cytometry and/or bacterial culture. In the beginning, a total of 215 urine samples were examined to study the optimal cut-off value for further bacterial culture by using a receiver operating characteristic (ROC) curve analysis, then the 7,550 urine samples were examined for evaluation of the cut-off values for further cultures. Samples were received within 2 – 4 hours from collection and processed immediately once delivered to microbiology laboratories.

Quantitative automated urinalysis system

The Sysmex UF-1000i performs an analysis of bacteria and leukocytes particles in urine by flow cytometry.[13-16] According to the manufacturer's guidelines, the 800 μL of each

sample was centrifuged at 400 g for 5 minutes, then the supernatant was discarded and the 200 μL of residual sediment was suspended. 10 μL of suspended pellet was loaded into FAST-READ chambers, and the leukocyte and bacterial particle counting results were expressed as WBC μL and BACT μL.

Microbiological analysis

Subsequently, samples were cultured by using calibrated loop onto blood agar medium and incubated at 35˚C for 18 – 24 hours. Based on preset validated threshold values, the amount of growth was scored as no growth, $< 10^3$ CFU/mL growth, 10^3 to 10^4 CFU/mL growth, 10^4 to 10^5 CFU/mL growth, and $\geq 10^5$ CFU/mL growth. Grown colonies were identified by their color on blood agar and a simple additional test in the case of *E.coli* (brown to burgundy on blood agar and indole test positive), *Proteus mirabilis* (clear brown on blood agar and indole test negative), and *Enterococcus spp.* (turquoise and growth on CAP blood agar). Other colonies were Gram stained and identified by standard methods when relevant (Gram-negative rods and Gram-positive cocci in groups by the Vitek-2 system [bioMerieux], hemolytic streptococci by Lancefield typing, and viridans streptococci by API-STREP [bioMerieux]).

Data analysis

The results of the Sysmex UF-1000i BACT and WBC counts were compared with the results of urine cultures using Receiver Operating Characte Sensitivity, specificity, positive predictive (PPV) and negative predictive value (NPV) were calculated for the two parameters at different cut-offs with respect to the reference urine culture test at a limit of 10^5 CFU/mL. Diagnostic accuracy of bacterial and WBC counts for UTIs was assessed by the area under the ROC curve (AUC). Statistical analysis was performed with the software SPSS 11.0 and GraphPad Prism for windows. The Student's t-test was applied to estimate imprecision to compare mean values ($p < 0.05$: statically significant differences).

Results

Receiver operating characteristic (ROC) curve analysis

This retrospective analysis included all adult patients who received UTI testing and had a suspected urinary tract infection. The goal was to determine the parameters of urine flow cytometry that may be used as a forecast for the development of mixed flora and urine culture. During this time, 215 samples were observed, of which 122 (56.7%) were culture positive (Fig. 1A) and 93 (43.3%) were culture negative (Fig. 1B). The receiver operating characteristic (ROC) curve study revealed a strong correlation of 0.83 for leukocytes and 0.94 for bacterial count (Fig. 1C). Table 1 displays the results at various cut-off values. When the findings of urine culture were compared to the numbers of bacteria and leukocytes detected in the urine sediment, it was discovered that the samples with positive urine culture results had more bacteria and leukocytes than the samples with negative urine culture results.

The combination of leukocytes ≥ 10 WBC μL and/or bacteria ≥ 100 BACT μL was found to be the most suitable cut-off value, which had 100% sensitivity, but only 22.6% specificity, which resulted in a 62.9% PPV and a 100% NPV, as shown in Table 1. Thus, we established a UTI screening package based on these criteria in the emergency medical order of E-Da Hospital. When the number of leukocytes in the urine sediment is less than 10 WBC μL and the number of bacteria is less than 100 BACT μL, urine culture is not required; however, when the number of leukocytes is ≥ 10 WBC μL or the number of bacteria is ≥ 100 BACT μL, urine culture is necessary. Detecting many culture-positive samples can be done by screening urine samples for UTIs.

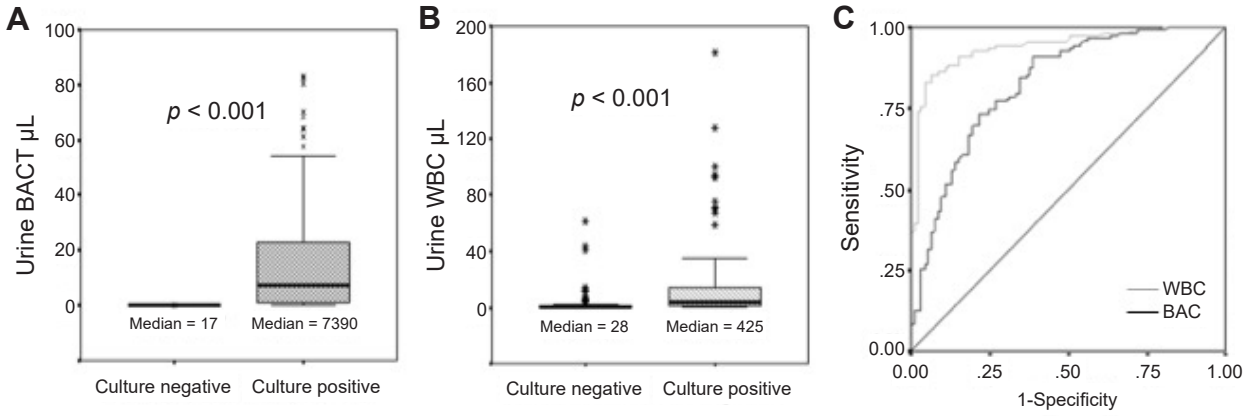

Fig. 1 Receiver operating characteristics (ROC) curve for bacteria and leukocyte counts on the Sysmex UF-1000i flow cytometer, using urine culture as the reference method (n = 215). (A) Urine bacteria ROC curve (p < 0.001). (B) Urine leukocytes ROC curve (p < 0.001). (C) Using ROC curve to analyze the relationship between the number of leukocytes and the number of bacteria in the results of urine culture. The area under the curve is 0.83 for leukocytes and 0.94 for bacterial counts (0.5 – 0.7: low accuracy, 0.7 – 0.9: moderate accuracy and > 0.9 high accuracy). ROC curve generated with SPSS Statistics V17.0.

Table 1. Performance of the Sysmex UF-1000i at different cut-off thresholds for leukocyte count and bacteria count.

	BACT µL	WBC µL	Sensitivity (%)	Specificity (%)	PPV	NPV
UF-1000i	100	-	91.0	82.8	87.4	87.5
	100	10	100	22.6	62.9	100
	100	50	96.7	53.8	73.3	92.5
	100	100	96.7	66.6	79.2	93.9

NPV: negative predictive value; PPV: positive predictive value.

Validation of the ideal cut-off parameters for predicting urine culture growth

The significance of the ideal cut-off criterion for further culture plating was then investigated using a total of 7,550 urine samples from the Emergency Department of E-Da Hospital. There were 2,862 samples (37.9%) among those 7,550 urine samples that did not have bacteria nor leukocyte counts below 10 WBC µL or 100 BACT µL respectively, in the urine sediment; hence no urine culture was required. Furthermore, there were 4,688 samples (62.1%) that had leukocyte counts ≥ 10 WBC µL or bacteria counts ≥ 100 BACT µL, of which 673 samples (14%) had negative urine culture results, and 2,510 samples (54%) had positive urine culture results (Table 2). The urine culture results revealed that 1,505 samples (32%), of which 711 samples exhibited staining and colony counts, 121 samples revealed the growth of several strains, and 673 samples were reported as contaminations.

Out of 4,688 total samples for further urine culture, there were 35% male and 65% female samples. The proportion of females who had a positive urine culture and suspected specimen contamination was significantly greater than that of males, while their male counterparts had significantly greater negative urine culture results, as shown in Table 2. The use of cut-off values for urinalysis can reduce a significant culture

Table 2. The sample number of leukocyte count on gender with a total of 4,688 urine samples by using cut off values to study 7,550 UTI samples from the Emergence Department of E-Da Hospital.

	Male	**Female**
Urine culture		
Culture-positive cultivation	703	1,807
Culture-negative	378	295
Culture-contamination	540	965

Culture-positive = bacterial growth > 10^3 cfu/mL.

negative rate from 43.3% (with 93 negative culture results from 215 total samples) to 8.9% (with 673 negative culture results from 7,550 total samples) and can improve a significant culture growth rate from 56.7% to 86%. This significantly reduces the workload and response time associated with culture plating, but there is still a 32 % growth rate associated with contamination in urine culture samples.

Correlation of urine sediment and gender in urine cultures of negative, positive and multiple strain groups

Among the 4,688 urine samples for further culture, 2,510 isolates of microorganisms were grown with 1,228 *E.coli* (48.9%), 165 *Pseudomonas aeruginosa*, 159 *Klebsiella pneumonia* (6.3%), 137 *Gardnerella vaginalis* (5.5%), 137 *Lactobacillus species* (5.5%), 111 *Enterococcus species* (4.4%), 70 *Proteus mirabilis* (2.8%), 59 *Corynebacterium species* (2.4%), which means that 673 samples were negative (14.4%) and 1,505 samples were suspected of contamination (32.1%) by urine culture (Fig. 2).

In 673 urine samples with negative culture, the number of urine sediment leukocytes in the range of < 10 and 10 – 28 WBC μL showed a significant row factor of $p^{**} < 0.006$ in both genders (Fig. 3A), and the number of bacteria in the range of < 100 BACT μL and 100 – 1,000 BACT μL showed a significant row factor of $p^{**} < 0.0125$ in both genders of culture-negative results (Fig. 3B). It suggests that the less leukocytes or bacteria count in flow cytometry, the higher the possibility of a negative urine culture.

In 2,510 urine samples with positive culture, the sample number in females are higher than in males ($p^{***} < 0.0007$), but there was a significant bacterial growth in samples with a leukocyte count > 556 WBC μL ($p^{**} < 0.0029$) (Fig. 4A) or bacteria count with 10^2 – 10^3 BACT μL, 10^3 – 6×10^3 BACT μL and > 6×10^3 BACT μL in females are higher than in males ($p^{*} < 0.0431$) (Fig. 4B). It suggested that the cut-off value of bacteria count > 10^2 BACT μL in urine samples can be used to predict further urine cultures. However, use of the cut-off value of leukocyte count > 10 WBC μL is not sufficient to support the need for further urine culture, leukocytes in urine may also be seen in inflammatory processes without underlying bacterial infection.

A total of 1,505 samples, including 965 samples from females and 540 samples from males, were suspected of being contaminated by urine culture. It was found that more samples from the female group had four mixed-type bacteria than the male group (Table 2). There are 711 contaminated growth samples that were reported, and the colony, leukocyte and bacterial counts in the urine sediment were used to categorize them. In both genders, Figure 5A showed that the number of leukocytes in the contaminated growth samples was significant with $p^{**} < 0.0032$ in the range of < 10 WBC μL, 10 – 28 WBC μL, 28 – 56 WBC μL and

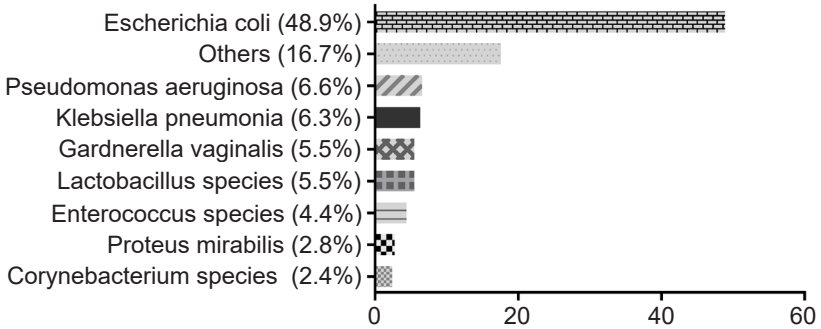

Fig. 2 The bacteria identification in 2,510 positive urine culture samples. Escherichia coli is the major stain found in urine culture positive group.

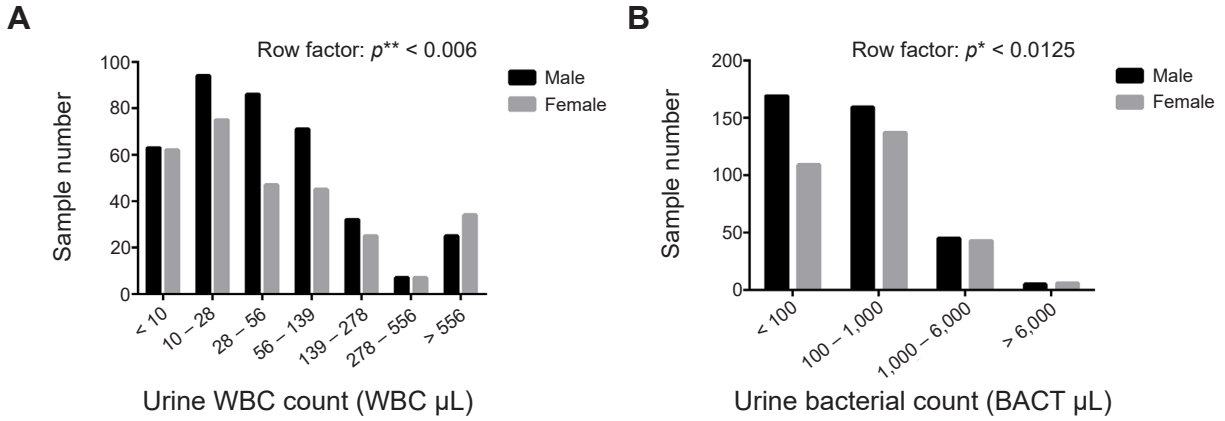

*Fig. 3 673 negative urine culture analysis. According to the results of urine sediment leukocyte and bacteria count by gender analysis, (A) the number of negative cultures with < 10 and 10 – 28 WBC μL samples are dominant in both gender (p** < 0.006), (B) among which < 100 BACT μL and 100 – 1,000 BACT μL samples are dominant in both gender (p* < 0.0125).*

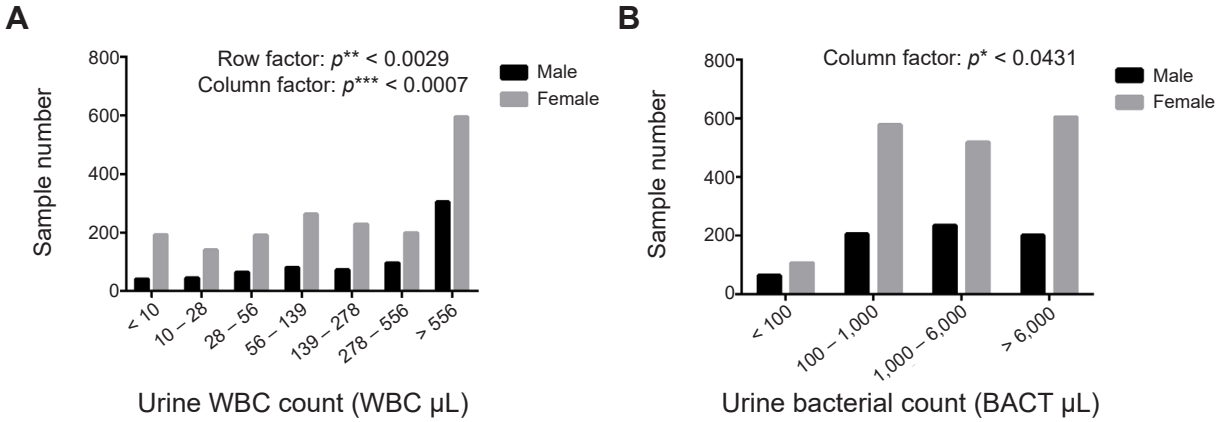

*Fig. 4 2,510 positive urine culture analysis. According to the results of urine sediment leukocyte and bacteria count by gender analysis, (A) the number of positive cultures in female is higher than in male (p*** < 0.0007) and among which urine sample with > 556 WBC μL is dominant in both gender (p** < 0.0029), and (B) the number of positive cultures with 100 – 1,000 BACT μL, 1,000 – 6,000 BACT μL and > 6,000 BACT μL in female is higher than in male (p* < 0.0431).*

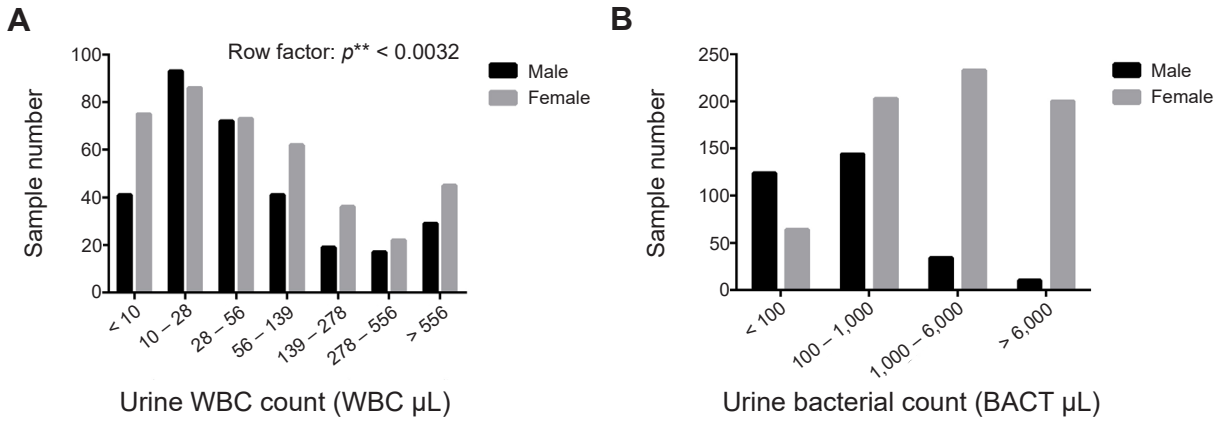

*Fig. 5 711 contamination urine culture analysis in WBC and bacteria count. According to the results of urine sediment leukocyte and bacteria count by gender analysis, (A) the number of culture contamination with < 10 WBC μL, 10 – 28 WBC μL, 28 – 56 WBC μL and 56 – 139 WBC μL samples are dominant in both gender (p** < 0.0032), (B) among which 100 – 1,000 BACT μL, 1,000 – 6,000 BACT μL and > 6,000 BACT μL samples are dominant in female.*

56 – 139 WBC μL. In Figure 5B, the number of bacteria in the contaminated growth samples was dominant in the female group with the range of 10^2 – 10^3 BACT μL, 10^3 – 6×10^3 BACT μL and > 6×10^3 BACT μL, but it was not found in male group. It implied that the use of cut-off predictors can exclude 2,862 samples from 7,550 urine samples for further plating, as a significant culture negative rate was reduced from 43.3% to 8.9%, and growth rate was increased from 43.3% to 86%.

Discussion

When bacteria or other pathogens, usually from fecal flora and enter the urinary tract it can induce a condition known as a UTI. The urinary system may experience effects from UTIs in several different regions, however, they can also damage the prostate, urethra, kidneys, and pyelonephritis. Cystitis is the most common UTI symptom (prostatitis) and it is essential for subsequent treatment to find and recognize bacteria in urine samples. Urinalysis is a routine screening test for all UTI suspected patients, making it one of the most frequently requested procedures in clinical laboratories. Traditionally, urinalysis is carried out utilizing a manual urine sediment inspection and a urine reagent strip test, however, modern technology now allows for automatic sediment analysis. These analyzers evaluate urine sediment using two analytical principles of image analysis and flow cytometry.[17] The Sysmex UF Series is an example of the other type that relies on flow cytometry and these analyzers can quickly and accurately count a wide variety of particles and classify them according to their size, shape, and other characteristics. As a result, they can forecast the outcomes of urine cultures and possibly eliminate pointless urine cultures without compromising positive urine cultures. In our study, the use of cut-off values to select urine samples for further culture plating by Sysmex UF-1000i did improve the positive culture rate, however there are high proportions with contamination. It had been reported that contaminated urine samples resulting in a mixed culture had a higher count of squamous cells than non-contaminated samples.[18] Flow cytometry parameters can safely predict a urine culture result without significant bacterial growth (rule out), on the other hand, positive urine culture growth (rule in) or even mixed culture growth suggesting contamination cannot be adequately predicted nor differentiated using flow cytometry parameters safely.[19] Some studies had demonstrated that the use of urinary dipstick reduced the urine laboratory workload,[20] and diagnostic value of dipstick test in adult symptomatic UTIs which showed a significant accuracy in positive results of leukocyte esterase and the nitrite, and it suggested that an alternate diagnosis should be considered if the leukocyte esterase is negative.[21] In order to be able to predict “rule out” and “rule in” cultures in the future, additional clinical parameters are necessary. Following the installation of the Sysmex UF-1000i in the clinical laboratory at E-Da Hospital, validation of the ideal cut-off for urine bacterial and leukocyte counts is necessary for the thousands of urine samples needed to diagnosis UTIs each month. Using Sysmex UF-1000i, urine samples with no or very few bacteria could be reported to the clinician up to 48 hours sooner than with standard culture. If sediment analysis could predict culture-negative samples, fewer additional laboratory tests would be necessary, and patients would not be a candidate for antibiotic therapy. The contamination in positive culture is an important issue that needed to be addressed and for the healthcare system, it allows for a more rapid diagnosis of the patient's condition, reducing both the number of patient bed-days in the hospital and the need for antibiotics.[4-9]

Conclusions

Our results demonstrate that flow cytom-

etry is a rapid, reliable, and effective screening technique that can significantly decrease the amount of unnecessary microbiological lab cultivations. As a result of the study's findings, we have implemented a quick, accurate, and reliable urine flow cytometry screening as a component of routine diagnostics for urine, increasing the sensitivity and specificity of UTI screening and drastically lowering the number of unnecessary cultivations in the microbiology lab at E-Da Hospital.

Author Contributions

Study Design, Li-Feng Liu, Chih-I Chen and Chen Chih-Hao; Data Collection, Ci-Meng Wang; Statistical Analysis, Chih-I Chen; Data Interpretation, Jia-Rong Cai, Pei-Chi Li; Manuscript Preparation, Felicia Leviana Suwandi, Joseph Sergio Kyler, and Kamal Lawrence Andrew; Literature Search, Franklin Chikodi Udo Kalu, Bing-Sian Lin, Meng-Han Tu, Hsing-Tung Yeh, Cyuan-Ya Yang, and Chung-Wei Yu.

Funding

This work was supported in part by grants from the E-Da Hospital (EDAHS108003), and Kaohsiung Municipal Min-sheng Hospital (KMSH-11003) Taiwan.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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