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Original Research Paper

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EVALUATION OF THE NEW SYSMEX UF-5000 FOR DETERMINATION OF CUT-OFF VALUES FOR LEUCOCYTES AND BACTERIA AND UTILIZING IN SCREENING OF URINARY TRACT INFECTION

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ABSTRACT Urinary tract infections (UTIs) represent the most frequently occurring infectious diseases in hospitals and community populations 1,2. Enteric bacteria remain the most frequent cause of UTIs, although the distribution of pathogens that cause UTIs is changing. Physicians distinguish UTIs from other diseases that have similar clinical presentations with use of a small number of tests, none of which, if used individually, have adequate sensitivity and specificity 3. Urinary tract infection is diagnosed on the basis of symptoms, signs, and urinalysis. For a final diagnosis, quantitative measurement of bacterial concentrations in the urine is mandatory. However, quantitative bacterial culture is very time consuming and expensive. Therefore, there is need of reliable screening method for differentiation of urine samples which contain significant numbers of bacteria from those which do not. Approximately 80% of urine cultures are negative 4. Automated analyser using flow cytometry has been recognized as capable of counting bacteria which have been utilized in predicting UTI 5, 6.

KEYWORDS : Bacterial count, Enterobacteriaceae, UF-5000, Urine Flow Cytometry, Urinary tract infection

INTRODUCTION

Urinary tract infections (UTIs) represent the most frequently occurring infectious diseases in hospitals and community populations (1,2). Enteric bacteria (in particular, Escherichia coli) remain the most frequent cause of UTIs, although the distribution of pathogens that cause UTIs is changing. More important is the increase in resistance to some antimicrobial agents, particularly the resistance to trimethoprim-sulfamethoxazole seen in E. coli. Physicians distinguish UTIs from other diseases that have similar clinical presentations with use of a small number of tests, none of which, if used individually, have adequate sensitivity and specificity (3).

Urinary tract infection is diagnosed on the basis of symptoms, signs, and urinalysis. For a final diagnosis, quantitative measurement of bacterial concentrations in the urine is mandatory. However, quantitative bacterial culture is very time consuming and expensive. Therefore, there is need of reliable screening method for differentiation of urine samples which contain significant numbers of bacteria from those which do not. Approximately 80% of urine cultures are negative (4). Automated analyser using flow cytometry has been recognised as capable of counting bacteria which have been utilized in predicting UTI (5, 6). In this study we used a fully automated Urine particle analyzer UF-5000 (Sysmex Corporation. Kobe, Japan) installed in our lab. The UF–5000 is based on Flow Cytometry and is a urine particle analyser for determination of clinical parameters in human urine & Body fluids. Quantitative values of analysis parameters (RBC, WBC, EC, CAST and BACTERIA) can be obtained using non-centrifuged samples (7).

The objective of this study to validate the performance & determine the cutoff value of leucocytes (WBC's) and bacterial count using fluorescence flow cytometry and utilizing for screening of UTI in patient population.

MATERIALS & METHODS

Patient &sample preparation

We evaluated 185 urine samples in the age group of 25 years to 88 years. Fresh midstream urine collected in sterile containers from walk in patients received in the laboratory for routine urine examination were analyzed for urine flowcytometry and bacterial culture. These samples were used for determining diagnostic cut offs for WBC and Bacteria for UTI diagnosis. Further 200 samples were processed to utilize the achieved cut-off in screening of UTI

Urine culture & Identification of Bacteria

The urine samples were analyzed for Urine culture and bacterial identification. The collected urine was inoculated onto 5% Sheep blood agar plate and MacConkey agar plate using a calibrated loop (10 L), according to the standard guidelines. Both agar plates were incubated at 35-37 °C for 18-24 h and inspected for colony morphology. Suspected fungi colonies were excluded (Candida). Colony counting was carried out and expressed as number of colony-forming units (CFU) per mL. For preliminary identification Gram staining was done which differentiated gram positive and gram-negative organisms. For identification of bacteria Biochemical tests were done. Gram negative organisms of more than 10^5CFU (Colony forming units) /ml and Gram-positive bacteria of more than 10^4CFU (colony forming units)/ml is indicative of UTI as per Domestic and National Committee for Clinical Laboratory Science standards for the diagnosis of UTI.

Sysmex UF-5000

UF-5000, the fully automated urine particle analyzer (Sysmex Corporation; hereinafter- 5000) is a new type of analyzer that can analyze the birefringence of particles and the amount of nucleic acid content and size information of the cell coupled with the complexity of internal structure, using a blue semiconductor laser (488 nm). With an improved optical system, detailed analysis of signal waveforms originating from each particle has been realized, and casts, epithelial cells, etc. can now be analyzed in greater detail the UF-5000 is very useful in the screening and identification of urinary tract infections (UTI). In addition, the new Bact Info-flag provides valuable information as to which antibiotic to prescribe to the patient with a UTI. So, UF-5000 offers a faster and far easier way than the classic Gram staining to provide the same

information. The sample volume required for analysis is 2 mL (0.6 mL for STAT mode) with an aspiration volume of 0.45 mL.

PERFORMANCE EVALUATION

Precision & Accuracy

The RBCs, WBCs, casts, bacteria, and epithelial cells using UF-Control samples were examined manually. The control was continuously examined 11 times. The results of the last 10 examinations were recorded and analyzed.

Carryover

Rinsing steps between samples were used in all analyses. In this mode carryover evaluation for bacteria was performed by measuring specimens with high values in triplicate, followed by a triplicate of specimens with very low values (blank). This series was consecutively analyzed three times. The carryover was determined by the formula: Carryover=(blank 1–blank 3)/(high 3–blank 3) for all three runs and mean values were calculated for each parameter.

Linearity

The linearity was calculated only for the main parameters (RBC, WBC & Bacteria). Aliquots with a final scalar concentration in a meaningful range (1, 10, 50, 100, 500, 1000, 5000, 10.000 L) were obtained by mixing a different proportion of physiological saline and a selected sample with a high cellularity (10.000 L or more). Each aliquot was repeated thrice, and the mean was calculated. A graphic representation of data was generated between measured and theoretical values and the linear regression coefficient, the slope and the intercept was calculated.

DATA ANALYSIS

Clinical history and culture result were collected daily and entered into pre-designed excel files. The bacterial count, WBC count. All of these excel files were merged together. Data were analyzed using MedCalc 19.0.5. The sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were presented for WBC cut-off value and bacterial counts cut-off value, against culture result as the reference. The receiving operator characteristics (ROC) curves for bacteria and WBC count were plotted to assess the cut-off values with Youden Index Method. The results of the study were presented as diagram figure and table with number and percentage.

RESULTS

UF-5000 for Identifying Gram-Positive and Gram- Negative Bacteria

Among another 200 urine culture specimens 53 specimens were negative for culture & 147 were found to be culture positive which comprises of 92% were gram-negative bacteria , 8% were gram-positive bacteria , and 0.7% was fungi. The most common microorganisms identified in a study from a urine sample (in order of frequency) were as follows: Escherichia coli, Klebsiella pneumoniae, Pseudomonas Spp, Acinetobacter Spp., and Citrobacter (gram-negative bacteria); Enterococcus Spp., Staphylococcus Spp. (gram-positive bacteria); and Candida albicans and (yeast). (Table 1).

Table 1. Bacteria Strain identifies from culture positive samples

Bacteria Gram-Negative	n	%	Bacteria Gram-Positive	n	%	Yeast	n	%
E. coli	106	72	**Staphylococcus**	1	0.7	**Candida Spp.**	1	0.7
Klebsiella spp.	25	17	**Enterococcus**	10	6.8			
Pseudomonas spp.	1	0.70						
Enterobacter	1	0.70						
Acinetobacter	1	0.70						
Citrobacter Spp.	1	0.70						

Performance Evaluation

Precision

Precision was calculated using the data obtained from 10 runs, from two levels of quality control samples performed. All the reportable parameters (RBC, WBC, EC, CASTS & Bacteria) showed an acceptable coefficient of variation (CV). In particular, the precision was 6.97%, 8.89%, 9.24%,20.68%,11.06% for a normal level control and 1.93%, 2.03%, 4.45%, 7.72%, 4.57% for a high-level control, respectively.

Accuracy

Accuracy was calculated using the data obtained from 10 runs, from two levels of quality control samples performed. All the reportable parameters (RBC, WBC, EC, CASTS & Bacteria) showed an acceptable Bias (%). In particular, the bias was -1.8%, -0.6%, +16.3%, -7.1%,+4.6% for a normal level control and -2.7%, -0.3%, +10.1%, +0.7%, +4.5% for a high-level control respectively.

Carryover

The carry-over of UF-5000 was evaluated with the specific formula on the parameters that are likely to be present in high number in the urine samples (RBC, BACT) and with a target< 0.05% ; it was found to be excellent for all the considered parameters, with complete absence of sample carry-over on UF-5000 (0.0 %) (Table 2)

Table 2. Results of carry-over tests

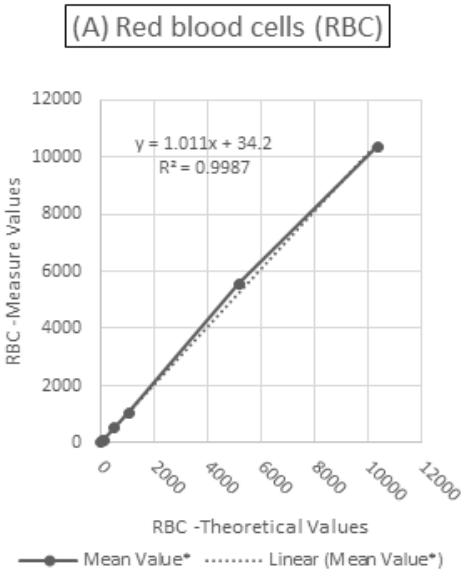
	High Value (mean)	Low Value (mean)	Carryover results (%)	Target (%)
RBC	10279.6	0.3	0.00	<0.05
WBC	10008.3	0.1	0.00	<0.05
BACT	10087.6	0.4	0.00	<0.05

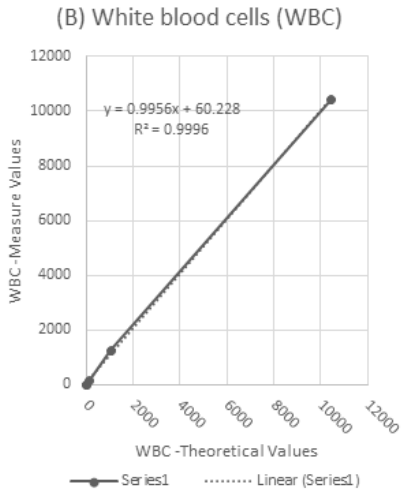
Red blood cells (RBC); White Blood Cells (WBC);Bacteria (BACT).

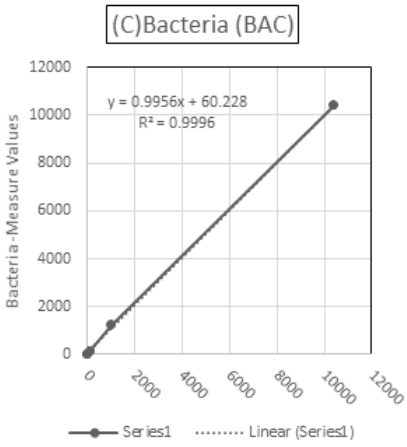
Linearity

Linearity was conducted in a wide range of value (approx. 0–10.000 for RBC, WBC & Bacteria and we obtained a linear regression coefficient of determination was 0.99, 0.99 and 1.00 respectively for RBC, WBC and Bacteria.

Figure 1. Linearity plot. (A) Red blood cells (RBC); (B) White blood cells (WBC); (C)Bacteria (BAC).



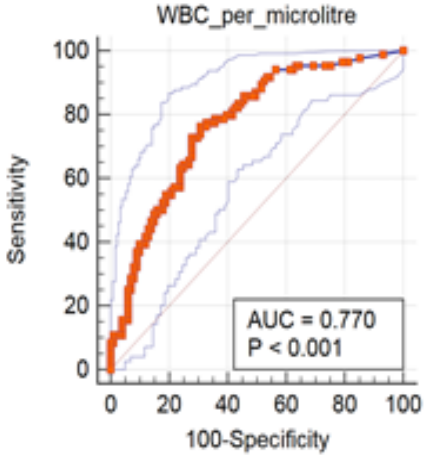


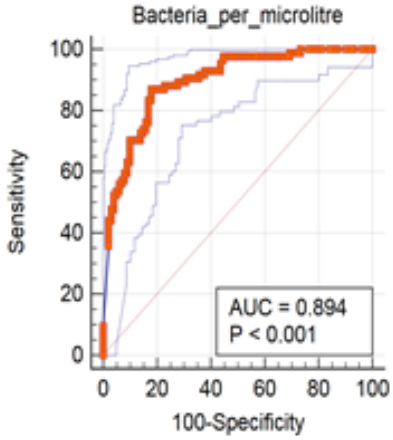


Bacteria and WBC Cut-off for UTI Screening

In this study, the bacterial count and WBC count showed the area under the ROC (AUC) of 0.89 and 0.77, respectively (Figure 2). According to the AUC, we used cut-off of bacterial count for ≥ 910.6 bacteria/μL and achieved sensitivity of 86.90% and specificity of 82.18%. The cut-off of WBC count was used at ≥ 61.1 cells/μL and achieved sensitivity of 76.19 % and specificity of 69.31 %.Using the combination of bacterial count ≥ 910.6 bacteria/ μL, WBC count ≥ 61.1 cells/μL, in 200 processed urine sample , achieved sensitivity of 99.32%, specificity of77.36 %, PPV of 92.36% and NPV of 94.62%. This combination showed positivity estimation of culture growth based on these criterions was 97.62%.

Figure 2A&B. ROC curve for UF-5000 bacterial count (BACT) and UF-5000 leucocyte count (WBC) versus urine quantitative culture in 185 specimens.





DISCUSSION

Urinary tract infection is a common disease but often neglected in daily clinical practices. Urine culture with semi-quantitative colony counting still remains gold standard for diagnosis UTI. However, since the urine culture needs time and technical experience, many clinicians still rely on patient symptoms and routine urine examination to diagnose the UTI (8).

Our study has shown the additional value of urine flow cytometry (WBC count and bacterial count) to improve the UTI diagnosis. For many years, the combination of nitrite and/or leukocyte esterase was common and widely used as screening of UTI, besides sign and symptoms in the clinical practices. A clinical useful screening method for UTI should be rapid, inexpensive, easy to perform and must have the highest values of sensitivity and NPV. This would mean a prompt reporting of normal samples, an improvement in the efficiency and quality of microbiological diagnoses reducing TAT, without losing valuable time in treating the patients. Moreover, the use of automation will allow large numbers of specimens to be processed with reduced technical labor.

The purpose of this study was to determine the optimal cut-off and utilizing it culture proven UTI. we evaluated the performance of SysmexUF-5000 in comparison with the urine culture method for screening urine samples for UTI. We considered, Gram negative organisms of more than 10^5CFU(Colony forming units) /ml and Gram-positive bacteria of more than10^4CFU(colony forming units)/ml is indicative of UTI as per Domestic and National Committee for Clinical Laboratory Science standards for the diagnosis of UTI. The selection of this criterion for the diagnosis of UTI was based on the heterogeneous patient population. We compared several studies from the last five years. In the last 5 studies, there were various cut-off value for bacteria ranging from 55 bacteria/μL to 288.9 bacteria/μL, and for WBC cut-off value, the range was from <24 cells/μL to 150 cells/μL (2, 9, 10, 11, 12). The possible explanations for those wide ranges were difference in study population and definition of UTI. Most of the studies population used laboratory-based method instead of clinical patient symptoms.

In our understanding from this study, the high specificity and PPV from this combination can be interpreted as the positive estimation of culture growth, which is achieved as high as 92.% among the study population. This means that result of positive estimation of culture growth gives an opportunity to urine flow cytometry to play a role in microbiology aspects, clinician decision-making and also patient management. However, false positive and false negative result can be seen for both bacteria and WBC count cut-off. The false result for WBC and bacteria count can be due to many factors, such as persistence of WBC after antibiotic treatment, dead bacteria or any debris that could be counted in the flow cytometer (4, 13) .The number of false positive cases could be reduced if the

information on clinical symptoms and antimicrobial assumption is obtained, indicating true infection at lower growth, thus enhancing specificity. In fact, in some cases which were positive at the screening ,the absence of growth could be explained by the presence of non-viable bacteria due to antibiotic treatment, which gets counted by UF-5000, or by the persistence of high WBC counts caused by a unsuccessful treatment. Our study has certain limitations. We did not derive Cut-offs specifically for a male & Female population . Results might differ in these groups. Nevertheless, our study suggests that the SysmexUF-5000 can serve as a useful tool to screen routine urine samples for culture in high volume laboratories.

CONCLUSION

A flow cytometric urinalysis analyzer performs the operation in a more time- and labor-saving manner than manual urinalysis. In addition, increased throughput and decreased microscopy review rate are advantages of this system. It also offers an opportunity to improve the standardization of basic urinalysis. With the defined criteria, the UF-5000analyzer provides a reliable information, in patient with UTI in terms of leucocyte and bacteria count, to clinicians prior to bacteriologic culture results

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