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Determination of Cut- off values of Bacteria and Leukocytes for urine analyzer FUS-1000 for the diagnosis of Urinary Tract Infection in adults

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ABSTRACT

AIM AND OBJECTIVE

The microscopic examination of urine is the most commonly performed screening tests in the clinical laboratory to diagnose and monitor patients with urinary tract infections (UTI's) as well as systemic conditions. Semi-quantitative urine culture by far is the 'gold standard' method for the diagnosis of UTI's, but it is time consuming and labor intensive. This study aims to study the analytical and diagnostic performance of the FUS-1000 (Changchun Dirui Industry, China), a urine automated analyzer, and define an optimal cut-off value of bacteriuria and leukocyturia in comparison to the gold standard of urine culture using VITEK 2 at a tertiary care centre in Gurugram.

MATERIAL AND METHODS

This is a cross sectional study performed on 476 urine samples during April- August 2025 submitted for culture and urine routine analysis with a preliminary diagnosis of urinary tract infection. The study was conducted at Clinical Pathology laboratory at Artemis hospital, Gurugram. The cut- off values by receiver-operating characteristic (ROC) curve technique, sensitivity and specificity were calculated for leukocytes and bacteria.

RESULTS

Among the 476 urine samples submitted for culture, 246 cultures (51.6%) were positive and 230 (48.3%) were negative. The best cut-off values obtained from ROC analysis were 27 cells /µL for leukocyturia with a 68.94% sensitivity, 65.15%, specificity, a positive predictive value of 65.85%, a negative predictive value of 68.26%, positive likelihood ratio of 1.98 and accuracy of 67%. The best cut-off for the number of bacteria is 69 cells / µL with a sensitivity of 70.66%, specificity of 70.97%, positive predictive value of 74.39%, Negative predictive value of 67%, positive likelihood ratio of 2.64

and accuracy of 70.8% . The area under the curve (AUC) for the leukocyte and bacteria were 0.74 (95% CI: 0.70-0.79) and 0.78 (95%CI: 0.73-0.82) respectively.

CONCLUSION

The cut- off of 27 cells /µL for leukocyturia gives only moderate diagnostic performance. Compared to leukocyturia, bacteriuria cut-off at 69 cells /µL performs slightly better. Both the parameters can be used as a screening threshold. The test should be interpreted clinically along with other parameters such as nitrite, leukocyte esterase rather than relied on alone.

KEY WORDS

Urinary tract infection, adults, leukocyturia, bacteriuria, FUS-1000

1. INTRODUCTION

UTI is one of the major public health problems in terms of morbidity, with an estimated > 400 million cases annually reported worldwide[1]. Regarding its prevalence, UTI is more common in women. The microscopic examination of urine is the most commonly performed screening tests in the clinical laboratory. It holds an important role in evaluating various components and characteristics of urine, thus helping in diagnosing and monitoring nephrological, urological and various systemic conditions[2, 3]. Microscopy-based urine particle analysis has greatly progressed over the past decades, enabling high throughput in clinical laboratories[4]. Automation and workflow simplification have led to mechanical integration of test strip readers and particle analysis in urinalysis. As the information obtained by urinalysis is complex, the introduction of expert systems may further reduce analytical errors and improve the quality of sediment and test strip analysis[4]. Semi-quantitative urine culture by far is the 'gold standard' method for the diagnosis of UTI's, but it is expensive, time consuming and labor intensive[3, 5]. Furthermore, it is often unnecessarily applied to negative samples. Different cut- off value for leukocyte and bacteria would help to reduce unnecessary culture[6].

The FUS-1000 hybrid urine analyzer is fully automated instrument which has the measurement principles based on flow imaging technique and photoelectric colorimetry[7]. Images are captured by high speed camera. Within a certain time, the FUS-1000 camera captures 650 frames of images containing visible component for each sample. All images are evaluated by a high quality image processing software which is able to detect and classify the urine particles.

To the best of our knowledge, there has not been any study from India on the establishment of cut off values for bacteria and leukocytes from FUS-1000 (Changchun Dirui Industry, China) in comparison to urine culture as the reference method. The study aimed to evaluate the analytical and diagnostic performance of FUS-1000 and assess the concordance between leukocyturia and bacteriuria with urine culture to carry out fast and reliable diagnosis of UTI.

2. MATERIALS AND METHODS

This is a cross sectional study which will be performed on all urine samples received from patients suspected of UTI from April- August 2025. This study will be performed at the Clinical Pathology laboratory of Artemis Hospital, Gurugram. The informed consent will be taken from all subjects. Each sample will be both cultured on CLED agar and run on fully automated urine analyzer.

INCLUSION CRITERIA

Around 5 ml midstream clean-catch urine samples will be taken in universal containers and transported to the clinical laboratory within 30 minutes of collection. All urine samples from outpatients and inpatients were included in the study.

EXCLUSION CRITERIA

Inadequate sample volume (<3ml) or sample received after 2 hours of collection will not be included in the study. Growth of ≥3 different colonies will be considered as contaminant and will be excluded from the study.

PROCEDURE

Spot midstream urine sample (5ml) will be collected in accordance with standard guidelines. (Detailed instructions for collection were provided) using a disposable, sterile universal container with a screw lid. The sample will be transported within 30 minutes of collection to the laboratory. A semi-quantitative urine culture will be performed by inoculating 1µL of urine onto CLED agar and streaking the entire plate surface. The agar plates will be incubated aerobically at 35ºC for 24 hours. Positive results were defined as the presence of colony forming units $\geq 10^5$ /mL. No growth was considered negative. Two or more isolates without a dominant pathogen was regarded as contamination. The VITEK 2 Compact (Biomeriux) was used for identification of microorganisms. For urine routine analysis, the sample was run on automated urine analyzer. Urine particles were detected and classified by FUS-1000. Leukocyte and bacteria were counted per high power field (HPF, 400 X magnification).

STATISTICAL ANALYSIS

Statistical analysis was carried out by SPSS version 21 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). To determine the best cut-off values, the receiver operating characteristic (ROC) curve technique for bacteria and leukocytes were performed. Positive predictive value (PPV), Negative predictive value (NPV) and accuracy rate at the best cut-off values for bacteria and leukocytes were also calculated considering the urine culture as the reference. A value of $p<0.05$ was considered as statistically significant.

3. RESULTS

Of 476 samples, 349 were females with a mean age of 27 years (Range 18-78 years) and 127 were males with a mean age 31 years (Range 18-82 years). Among the 476 urine specimens submitted for culture, 230 cultures (48.3%) were negative, while significant microbiological growth was detected in 246 (51.6%) specimens. Escherichia coli were found to be the most common pathogen (65%) in our study.

The ROC curves for bacteria and leukocytes are depicted in Figure 1, in which culture results $\geq 10^5$ CFU/ml were taken as the reference for significant bacteriuria. The area under the curve (AUC) for the bacteria and leukocytes were 0.78 (95% CI: 0.73-0.82) and, 0.74 (95% CI: 0.70-0.79) respectively. There was no significant difference between AUC's of bacteria and leukocytes.

The best cut- off values obtained from the ROC analysis were 27 cells /µL for leukocyturia with a 68.94% sensitivity, 65.15%, specificity, a positive predictive value of 65.85%, a negative predictive value of 68.26%, positive likelihood ratio of 1.98 and accuracy of 67%. The best cut-off for the number of bacteria is 69 cells / µL with a sensitivity of 70.66%, specificity of 70.97%, positive predictive value of 74.39%, Negative predictive value of 67%, positive likelihood ratio of 2.64 and accuracy of 70.8% .

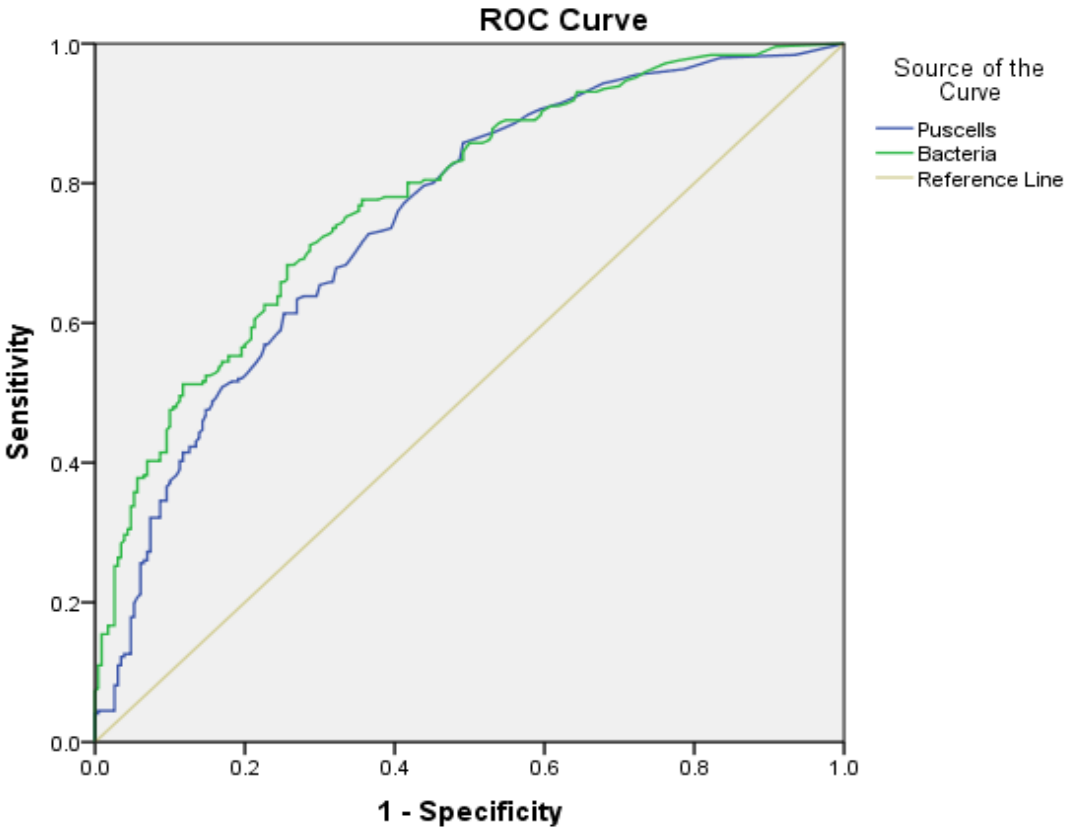

Figure 1: Receiver operating characteristic curves for pus cells and bacteria cut-off values

	Cut-off value of Leukocyte 26.5 cells/µL	Cut-off value of Bacteria 69 cells/µL
Sensitivity	68.94%	70.66%
Specificity	65.15%	70.97%

Table 1: Sensitivity and Specificity for Leukocyte and bacteria at cut-off values

	Leukocyte cut-off 26.5 cells/µL	Bacteria cut-off 69 cells/µL
Sensitivity (95% CI)	68.94% (63.16 -74.72)	70.66% (64.98 – 76.34)
Specificity (95% CI)	65.15% (59.0 – 71.4)	70.97% (65.0- 76.7)
PPV (95% CI)	65.85% (59.9 – 71.8)	74.39% (68.9 – 79.8)
NPV (95% CI)	68.26% (62.3 – 74.3)	66.96% (60.9 - 73.1)
Positive Likelihood Ratio (95% CI)	1.98 (1.64 – 2.41)	2.64 (1.96 – 3.05)
Accuracy	67.01%	70.80%

Table 2: Sensitivity, Specificity, PPV, NPV, Accuracy, and likelihood ratios for leukocyte and bacteria

4. DISCUSSION

The FUS-1000 is a new automated hybrid urine analyzer for identification and counting of bright field digital images of the formed elements in urine, however the urine strip chemistry component operates on the principle of photoelectric colorimetry. It is crucial to minimize false- negative results, particularly in immune-suppressed and elderly patients due to the potential risk of severe urinary sepsis. Conversely, a high rate of false positives should also be avoided, as it may lead to unnecessary antibiotic use or misinterpretation caused by the presence of normal Gram- positive flora. Overtreatment can further promote the survival of resistant bacteria that persist in small clusters, potentially leading to recurrent urinary tract infections (UTI).[8]

In this study, we evaluated the performance of FUS-1000 in comparison with the urine culture for screening urine samples for urinary tract infection (UTI).We established the optimum cut-off values for bacteria and leucocytes in our setting for the diagnosis of bacteriuria, assuming a cut-off $>10^5$ CFU/mL as significant bacteriuria for culture test. Possible false-negative cultures could be caused by the presence of dead bacteria in the urine due to treatment or a low bacterial load.

From the ROC (Fig1), the area under the curve (AUC) for the bacteria and leukocytes were 0.78 (95% CI: 0.73-0.82) and, 0.74 (95% CI: 0.70-0.79) respectively. There was no significant difference between AUC's of bacteria and leukocytes. Tables 1 and 2 show the best cut- off values obtained from the ROC analysis as 27 cells /µL for leukocyturia with a 68.94% sensitivity and 65.15%, specificity. This cut-off also gave a positive predictive value of 65.85%, a negative predictive value of 68.26%, positive likelihood ratio of 1.98 and accuracy of 67%.

In contrast to this study, Derya et al reported a higher cut-off of 34/µL with a sensitivity of 72.3% and specificity of 65.2% for pus cells on FUS-200[9]. Similar research by Bargotya et al reported a lower cut-off value of 19.8 cells/µL with a sensitivity of 82.3%, specificity of 80.9%, PPV of 54.3% and NPV of 93.5%[10]. Giesen *et al.* reported that the flow cytometry method resulted in a lower cut-off of leukocyturia (≥ 31.8 cells/µL) with a sensitivity of 89%, a specificity of 79%, PPV of 38%, and NPV of 98%[11]. Agpaoa *et al*. obtained cut-off of ≥ 27 cells/µL for leukocyturia, with a sensitivity of 84.6%; specificity of 65.4%; PPV of 71% and NPV of 81%[12]. The cut-off value for pus cells obtained in this study was similar to that of Agpaoa *et al*.

The best cut-off for the number of bacteria is 69 cells / µL with a sensitivity of 70.66%, specificity of 70.97%, positive predictive value of 74.39%, Negative predictive value of 67%, positive likelihood ratio of 2.64 and accuracy of 70.8% . Bargotya *et al*. determined a higher cut-off of 198.8 cells/µL with a sensitivity of 82.1%, a specificity of 79.5%, a PPV of 6 54.3%, and NPV of 92.7%[10] whereas Millan M et al obtained a cut-off of 89.4 cells/ µL with a sensitivity of 94.8%, specificity of 69.2% and NPV of 99.4% on UF-1000i urine analyzer[8].

Variations in the cut-off values of leukocyturia and bacteriuria may arise due to differences in study populations, specimen types, and the thresholds defined for significant culture counts. Therefore, each laboratory should determine and report its own reference values. Furthermore, since different automated analyzers may use varying analytical methods and thus produce different cut-off limits for bacteria and pus cells, clinicians must be aware of the specific system used in the laboratory and interpret urine analysis results accordingly, based on analyzer's established cut-off values.

5. CONCLUSION

In the present study, the main purpose of screening urine specimens was to find out if it is possible to predict positive cultures and thereby eliminate the negative cultures rapidly and safely.The cut- off of 27 cells /µL for leukocyturia gives only moderate diagnostic performance. Compared to leukocyturia, bacteriuria cut-off at 69 cells /µL performs slightly better. Both the parameters can be used as a screening threshold. The test should be interpreted clinically along with other parameters such as nitrite, leukocyte esterase rather than relied on alone.

According to the manufacturer, the FUS1000 is capable of detecting and classifying erythrocytes, leukocytes and squamous epithelial cells. For other urinary elements, it is advisable that trained personnel review the on-screen images for a definitive interpretation. With further technical advancements and enhanced staff training, the analyzer's performance is likely to improve and may show better results.

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